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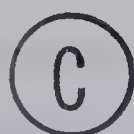
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Simultaneous Recovery Of Oil And Protein From Rapeseed By  
Aqueous Alkaline Extraction

by



MAHGOUB NAGY YEHYA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE  
OF MASTER OF SCIENCE

IN

FOOD SCIENCE

EDMONTON, ALBERTA

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THE UNIVERSITY OF ALBERTA  
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Simultaneous Recovery Of Oil And Protein From Rapeseed By Aqueous Alkaline Extraction submitted by MAHGOUB NAGY YEHYA in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in FOOD SCIENCE.





## Abstract

The objective of this work was to investigate the possibility of using one or two step aqueous-alkaline extraction for the recovery of oil and protein from rapeseed. The final suggested two step aqueous-alkaline process resulted in three products: good quality oil, protein concentrate and meal.

Prior to the selection of the final extraction conditions of the two step extraction, several factors of importance were investigated. Among these were factors affecting the first step, i.e. the aqueous extraction at the natural pH of the rapeseed to remove as much oil and glucosinolates as possible; factors affecting the alkaline extraction of preheated full-fat rapeseed to maximize protein solubility and oil extractability; and the effect of different combination treatments of heat and pH on the constituents of rapeseed, especially protein, fat, and glucosinolates.

The investigations indicated that in order to maximize protein solubility and oil extractability in the liquid fraction, pH 11 and high temperature (70°C) had to be employed. However, this treatment caused an increase in the free fatty acid content, protein discoloration, changes in amino acid pattern, production of lysinoalanine and changes in the gel electrophoresis pattern. Less severe treatments (25°C at pH 11 and 50°C at pH 10 or lower) produced little



measurable change in these quality parameters.

The results of the aforementioned investigations were used to suggest a two step aqueous-alkaline extraction procedure. The selection of the extraction conditions compromised the yield in favor of oil and protein quality.

In the first step, the enzyme deactivated heated ground rapeseed was blended for ten minutes before mixing for another ten minutes at natural pH of the rapeseed slurry (approximately 5.8), 80°C and solid to water ratio of 1 to 3.5. The mixed rapeseed slurry was centrifuged to produce liquid and solid fractions. The liquid fraction contained about 52% of the total oil-mostly in free state and most of the recoverable glucosinolates. The second step consisted of mixing the dispersion of the solid fraction for 30 minutes at pH 11 and 25°C to extract further 35.5% oil and 38% protein as a liquid fraction. Upon drying and oil removal by solvent extraction, bland, light colored protein concentrate (containing 69% protein) was produced. The residual meal contained 46% of the total protein and 15% of the total oil.

This simple two step aqueous-alkaline procedure can be used to replace some or all steps used in the conventional prepress-solvent extraction method. Alternatively, the first step can be used on its own right to replace the pressing step in the prepress-solvent extraction. The solid fraction could be either dried and solvent extracted for oil and meal, or alkali extracted for oil, protein concentrate and meal. The yields obtainable by this procedure could be



further improved by optimization of the engineering unit operations selected.





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## 1. INTRODUCTION

Rapeseed is Canada's most important oilseed crop. It is the fourth largest grain crop in Western Canada and Alberta (Kondra, 1977). Its importance is not only for its potential in the production of edible and industrial oils but also in the manufacture of high protein meals suitable for supplementing animal diets, and possibly high quality protein for human consumption. Rapeseed ranks fifth among the major oilseeds of the world, just after soybean, sunflower seed, oil palm, and peanut (Downey *et al*, 1974; FAO, 1980).

The earliest direct reference to the oilseed known as rape or sarson is found in ancient Indian sanskrit writing about 2000-1500 B.C. (Singh, 1958). The history of rapeseed in China is unknown, however, ancient Japanese literature indicates that rape was introduced to Japan 2000 years ago directly from China or via the Korean Peninsula (Downey, 1965). It is assumed that two rapeseed species had been cultivated as an oil crop for a long time in those parts of Europe where the olive and the poppy were unknown, though it was never used by the Romans (Appelqvist, 1972b). In the latter part of the middle ages it was the most important lamp oil in Europe north of the Alps until it was replaced by petroleum oil (Appelqvist, 1972b). The outbreak of World War II markedly changed the production pattern of rapeseed in several parts of the world. In Europe the total production in 1948-1952 was three to four times greater than



that during 1934-1938 (Appelqvist, 1972b).

Rapeseed was first introduced to Canada in 1942 as a war-time measure to supply oil for industrial purposes (Downey *et al.*, 1974). Its potential use as an edible oil for Canada was proposed in 1948 which led to the first domestic commercial extraction of edible rapeseed oil in Canada in 1956-1957 (Genser and Eskin, 1979). From this modest beginning, Canada has become, over the last ten years, the largest producer (Ohlson and Anjou, 1979) and the largest exporter of rapeseed in the world (Downey, 1976) as indicated in Table 1.

The contribution of rapeseed oil to the Canadian production of deodorized oils used in margarine and shortenings grew from a negligible amount in 1950 to 32.7 percent in 1975 and 36.3 percent in 1977 (Genser and Eskin, 1979). However, the high quality rapeseed protein has not been fully utilized yet.





Table 1. World production and net rapeseed exports\* by major producers

	Production				Exports
	1935-39 <sup>1</sup>	1945-49 <sup>1</sup>	1975-76 <sup>2</sup>	1977-78 <sup>2</sup> Forecast	Average <sup>3</sup> 1970-73
Canada	-	12	1749	1900	1066
China	2102	3100	1350	1350	160
India	969	1001	1936	1600	0
Pakistan			267	?	0
Poland	-	-	726	672	-
Sweden	-	-	285	233	-
West Germany	-	-	199	282	-
France	-	-	532	421	-
Western Europe					552
Eastern Europe	241	343			332

SOURCES:

1. Commonwealth Economic Committee 1936-64, Her Majesty's Stationery Office, London.
  2. Ohlson and Anjou, 1979.
  3. Downey, 1976.
- \* in 1000 Ton.



## 2. LITERATURE REVIEW

### 2.1 BOTANY OF RAPESEED

Oilseeds called by the name rapeseed do not represent one species. There are several species involved generally belonging to the genus *Brassica*. Sometimes industrial rapeseed will be a mixture of two or more types of species (Bengtsson *et al.*, 1972). A survey of the species from which commercial rapeseed may be derived is given in Table 2 (Bengtsson *et al.*, 1972). These species are related and are rather similar in appearance. They are also divided into subspecies, formae and varieties or cultivars (Bengtsson *et al.*, 1972). Turnip, rutabaga, cabbage, brussel sprouts, mustard, and many other well known vegetables are close relatives of the different rapeseed varieties (Downey *et al.*, 1974).

The rapeseed varieties currently grown in Canada are of the Argentine (*Brassica napus*) or Polish (*Brassica campestris*) species. Present cultivars within these varieties are low in both erucic acid and glucosinolates which is the result of deliberate plant breeding programs. Most of these cultivars are referred to in the Canadian rapeseed industry as canola (Genser and Eskin, 1979).



Table 2. Definition of Rapeseed<sup>1</sup>

Botanical (Latin) name	Correct English	Synonyms	French	German
<i>Brassica napus</i> ssp. * <i>oleifera</i> forma <i>biennis</i> forma <i>annua</i>	Winter rape Summer rape	Oil rape, rapeseed <sup>2</sup> swede rape oilseed rape	Colza d'hiver Colza de printemps Colza d'ete	Winterraps Sommertraps
<i>Brassica campestris</i> ssp. * <i>oleifera</i> forma <i>biennis</i> forma <i>annua</i>	Winter turnip rape Summer turnip rape	Rapeseed <sup>3</sup> oil turnip	Navette d'hiver Navette de printemps Navette d'ete	Winterrubsen Sommerrubse
forma <i>annua</i> var. <i>chinensis</i>	Summer turnip rape	Chinese mustard	Moutarde chinoise Pak-choi	Chinasenf
var. <i>pekinensis</i>	Summer turnip rape	Celery cabbage	Chou chinois Pet-gai	Chinakohl
var. <i>dichotoma</i>	Summer turnip rape	Toria	Toria	Toria
var. <i>trilobularis</i>	Summer turnip rape	Sarson	Sarson	Sarson
<i>Brassica juncea</i>	Brown mustard	Leaf mustard Indian mustard	Moutarde brune	Brauner Senf Sarepta Senf

\*subspecies

<sup>1</sup>From Bengtsson et al., 1972<sup>2</sup>Known in Canada as Argentine type<sup>3</sup>Known in Canada as Polish type





## 2.2 CHEMICAL CONSTITUENTS, NUTRITIONAL VALUES AND USES

The moisture content of 'naturally dry' rapeseed, (i.e., when the water in the seed is in equilibrium with the atmospheric moisture) generally ranges from 6-8%. Major components in dry rapeseed are lipids (oil), protein, carbohydrates and glucosinolates. There are other minor components such as sinapine, flavonoids, ash minerals and vitamins (Appelqvist, 1972a). There are often marked differences in composition between different cultivars or line of species (Sosulski and Bakal, 1969). The greatest variation is noted in glucosinolates (Clandinin *et al.*, 1978). The oil content of seeds of *Brassica napus* and *Brassica campestris* as determined by extraction with hydrocarbons varies over a wide range. Typical figures for winter rape are 40-48% and for summer rape 36-46% in dry matter (Appelqvist, 1972a). The residue from the hydrocarbon extraction of rapeseed is called rapeseed meal and the protein content of the meal varies from 40.8-44.4%. The crude fibre, mainly cellulose and hemicellulose, varies from 12.6-16.7%, ash from 7-8.4%, and nitrogen-free extract from 34.1-36.7% (Sosulski and Bakal, 1969). The high crude fibre content in rapeseed is a consequence of the small seed size which has a large proportion of seed coat (Appelqvist, 1972a). The amount of hull and the composition of the hull and meats from two varieties of rapeseed are shown in Table 3.





Table 3. Amount of hull and composition of hull embryos ('meats') from seeds of rape and turnip rape.

Constituent	<i>Brassica napus</i> cv Tanka(1)	<i>Brassica campestris</i> cv. Arlo(1)	<i>Brassica napus</i> cv. Oro(2)
Hull (per cent)*	16.5	18.7	17.7
Oil*			
Seed	41.5	40.0	41.4
Hull	16.0	16.2	
Meats	47.1	45.0	
Protein**			
Seed	44.7(a)	44.2(a)	43.0(b)
Hull	18.7	20.6	
Meats	53.6	53.4	49.4
Crude Fibre**			
Seed	11.8	11.7	
Hull	34.3	31.6	
Meats	3.0	3.6	

\* Moisture-free basis

\*\*Moisture-free, oil-free basis

a. Protein (NX6.25)

b. Protein (NX5.7)

SOURCES:1. C.G. Youngs, 1967.

2. Recalculated from Anjou et al, 1977. Oro is a low erucic acid low glucosinolates cultivar.



### 2.2.1 Lipids

Triglycerides generally account for 95-98% of the total lipids of mature *Brassica* seeds. The amount of mono- and diglycerides is very low representing less than 2%. The total amount of phospholipids and other polar lipids is rather low, representing about 0.5-1% in mature seeds (Appelqvist, 1972a).

The introduction of low erucic acid rapeseed oil markedly affected its chemical and physical properties. Current Canadian literature on edible rapeseed oil is concerned solely with the low erucic acid varieties (Genser and Eskin, 1979). Canola is now being used in the food industry for products such as salad oils, dressing, mayonnaise, cooking oil, margarine and shortening. Numerous reviews on the uses and nutritional aspects of rapeseed oil are available (e.g., Rocquelin *et al.*, 1971; Runer and Ohlson, 1971; Downey *et al.*, 1974; Beare-Rogers, 1975; Vles, 1974; Runer and Honkanen, 1972; Jorgensen, 1972; and Genser and Eskin, 1979).

Rapeseed is unique in that it contains a high amount of sulfur in comparison with other oilseeds. During the extraction of the oil, small amounts of glucosinolates could be hydrolyzed into isothiocyanates, oxazolidinethione and other sulfur compounds which are soluble in oil and can poison the catalyst during the hydrogenation process (Persmark, 1972; Sosulski, 1974).



### 2.2.2 Proteins and Amino Acids

The protein content of rapeseed varies depending on such factors as variety (Clandinin and Bayly, 1963) and environmental conditions (Wetter *et al.*, 1970). Rapeseed meal (RSM) contains about 40% protein (Appelqvist, 1972a). Of the total nitrogen, about 9% was reported to be non-protein nitrogen (Quinn and Jones, 1976). The contents of peptides and free amino acids in the non-protein nitrogen of rapeseed meal amounts to 7.2% and 13.3% respectively, or 2.4% of the meal weight (Rutkowski and Kozłowska, 1979). Studies on characteristics of rapeseed meal protein indicate that there are two major protein fractions obtainable by salt extraction: a neutral protein (12 S protein) and a basic protein (1.7 S protein) (Bhatty *et al.*, 1968., Finlayson *et al.*, 1969). The 12 S protein acts as a single molecule at pH values of 7.5-9 but dissociates into 7S components at pH 3.6 and 3S components at pH 2.2 (Finlayson *et al.*, 1969). Quinn and Jones (1976) reported that the rapeseed contained over 30 protein species and the majority of these proteins have isoelectric points in the neutral pH region. In another study, Janson *et al.* (1971) observed that the rapeseed proteins were composed of about 20 weak acidic proteins, about 20 neutral proteins and 4-6 basic ones. The water extract contains mainly acidic and neutral proteins, whereas the HCl solution (pH 2) extract is made up of mainly basic proteins (Kodagoda *et al.*, 1973a). The basic proteins account for 20% of the total soluble protein and have







molecular weight in the range of 15,000-20,000 (Janson *et al.*, 1971). About 5% of the soluble protein has molecular weight of 50,000-75,000 and the bulk of the protein has molecular weight from about 120,000 to 150,000 (Janson, 1971; Janson *et al.*, 1971). Different molecular weights of protein species were reported by other scientists (Chamnanwej, 1971; Kodagoda *et al.*, 1973a; Ohlson and Tear, 1974; Quinn and Jones, 1976). The nutritional value of a food or feed is dependent on several factors: protein content, amino acid composition, crude fibre content, mineral content, vitamins and the possible presence of toxic substances. As shown in Table 4, the protein content of rapeseed meal (RSM) is less than that of soya meal, however, it represents the most concentrated vegetable protein feed produced in the cool temperate zone (Josefsson, 1972). The amino acid content in the protein of RSM, as shown in Table 4, compares favorably with that of soybean meal (SBM); the latter contains more methionine. According to the provisional pattern of FAO, 1957, isoleucine and methionine are the limiting amino acids of rapeseed. RSM and SBM tend to supplement each other (Josefsson, 1972). Research by Sosulski and Sarwar (1973) showed that soybean and rapeseed proteins contained a higher proportion of essential amino acids than flax, sunflower and safflower proteins. Rapeseed proteins also have a higher level of methionine, cystine and proline, while soybean proteins had a higher level of phenylalanine. High ratings were given to soybean and



Table 4. Composition of rapeseed meal and soybean meal

	High Glucosinolate Prepress Solvent Rapeseed Meal (%)	Low Glucosinolate Prepress Solvent and Solvent Rapeseed Meal	Solvent Soybean Meal
	a	b	c
Moisture	8.78	7.47	11.00
Crude fibre	12.64	11.09	6.00
Ether extract	1.80	3.78	0.90
Protein (NX 6.25)	36.60	37.96	45.01
<i>Amino acids</i>			
Alanine	4.37	4.56	4.20
Arginine	5.60	5.82	6.44
Aspartic acid	6.88	8.03	11.20
Cystine	0.59	1.23	0.65
Glutamic acid	17.54	16.69	18.00
Glycine	4.92	4.96	4.60
Histidine	2.68	2.72	2.40
Isoleucine	3.72	3.98	4.69
Leucine	6.80	6.97	7.49
Lysine	5.65	5.57	6.22
Methionine	1.90	1.78	1.40
Phenylalanine	3.82	4.01	4.89
Proline	6.15	7.00	4.89
Serine	4.32	4.39	5.00
Threonine	4.31	4.50	3.80
Tryptophane	1.20	1.16	1.20
Tyrosine	2.20	2.46	2.80
Valine	4.86	5.11	5.00

a. Average of 29 samples.

b. Average of 7 samples of Tower rapeseed meal and 3 samples of Candle RSM.

c. protein and amino acid values represent averages of 6 samples.

SOURCE: From Clandinin *et al.*, 1978.



rapeseed meal and isolates. However, soybean was deficient in sulfur-containing amino acids, while the rapeseed meals were low in isoleucine. Since isoleucine deficiency is uncommon in a normal diet, Sosulski and Sarwar (1973) concluded that the rapeseed products appeared to have the highest quality as a protein supplement for humans.

### 2.2.3 Carbohydrates

Except for glucosinolates, the carbohydrates in rapeseed and turnip rapeseed have not been fully characterized (Theander and Aman, 1974). The amounts of the major low-molecular carbohydrates in rapeseed and soybean, determined by different workers, are presented in Table 5. The data indicate that sugars are present in rapeseed in significant quantities. Sucrose is the principal sugar, followed by stachyose and a limited amount of other sugars. Seasonal and other variations may perhaps explain the observed differences in the table #5. Stachyose and raffinose are frequently claimed to have intestinal gas-forming properties (Theander and Aman, 1974). In the industrial production of protein concentrates the low-molecular sacharides as well as the glucosinolates are removed by water extraction (Tape *et al.*, 1970; Ballester *et al.*, 1970a., Eklund *et al.*, 1971).

Insoluble polysaccharies (crude fibres) are mainly concentrated in the hull, which, depending on the variety and shape of the seeds, accounts for 12-20% of the seed





Table 5. Content of sugars in rapeseed and soybean.\*

Constituent	Rapeseed				Soya	
	a	b	c	d	e	f
Fructose	0.51	0.15	0.17	0.51	0.04	0.46
Glucose	0.21	0.28	0.24	0.40	trace	trace
Sucrose	1.11	2.26	7.10	7.49	6.94	3.99
Raffinose	0.15	0.32	0.33	0.31	0.41	0.74
Stachyose	0.19	1.52	2.47	2.39	2.39	6.26

\* % Dry basis

a. Mizuno 1958 (*B. napus*).

b. Siddiqui *et al.*, 1973.

c. Theander and Aman, 1974 (*B. napus* var. Bronowski).

d. Theander and Aman, 1974 (*B. campestris* var. Duro).

e,f. Kozłowska *et al.*, 1979





weight (Rutkowski and Kozłowska, 1979). The crude fibre of rapeseed is considerably higher than soybean (Ohlson, 1972) and peanut meal (Rosen, 1958), but similar to that of sunflower (Klain *et al.*, 1956). Cellulose, lignin and hemicellulose are the main polysaccharides in rapeseed. Their presence makes it impossible to obtain the customary protein concentrate (70% or more protein) using classical methods (Rutkowski and Kozłowska, 1979). The utilization of commercial rapeseed meal can be enhanced if the crude fibre content is reduced. This can be done by dehulling the seeds and by plant breeding (Ohlson, 1973). The hull can be removed by dry dehulling before oil extraction (Stanley and de Man, 1974; Ohlson, 1973), wet dehulling after diffusion extraction (Sosulski, 1974), or air classification of the meal (Tape *et al.*, 1970).

The introduction of the yellow seeded cultivars can reduce the crude fibre significantly (Stringam *et al.*, 1974; Stringam and Harvey, 1973; Kondra, 1979). According to Kondra (1977), "pure yellow-seeded rapeseed produces a meal with fibre content approaching that of soybean meal."

#### 2.2.4 Minerals and Vitamins

Ash content of rapeseed meal was reported to be from 5.8-6.6% for meals from Chile (Ballester *et al.*, 1970a), and 7-8% for the meals from Sweden (Ohlson, 1972). Clandinin *et al.* (1978) studied the ash and vitamin content of both RSM and SBM. The study showed that rapeseed meal has a higher



content of Ca, Fe, Mg, Mn, P, Se and Zn than soybean meal. Studies of the differences between meals from rapeseed of low and high glucosinolates showed that the first was very significantly lower in S and K but higher in P, Ca and B (Josefsson, 1972). Other differences were not significant (Kjaer, 1960). Clandinin *et al.* (1978) found that RSM has a higher proportion of the vitamin B group than SBM. RSM contains choline, folic acid, niacin, riboflavin and thiamine, all in higher amounts than of SBM.

#### 2.2.5 Other Constituents

Tannin, phytic acid, and sinapine are minor but important components of rapeseed (Rutkowski and Kozłowska, 1979). Yapar and Clandinin (1972) proved that rapeseed contains tannin. The content was found to vary from 2.6% (Fenwick and Hoggan, 1976) to 3% (Clandinin and Heard, 1968). Tannins bind with proteins and have shown to have an inhibitory effect on the digestive enzyme trypsin.

The rapeseed protein concentrate contains about 5% phytic acid (Hermansson *et al.*, 1974). The presence of phytic acid in protein extracts strongly affected the nitrogen recovery during alkaline extraction (Gillberg and Tornell, 1976). Phytic acid contained in rapeseed did not cause a reduction in the intestinal absorption of calcium, zinc and iron in rats fed on a diet containing rapeseed protein concentrate (Eklund and Agren, 1974). In vitro experiments have shown that phytic acid forms poorly soluble





salts with zinc, especially in the presence of calcium (Ohlson and Anjou, 1979). In rat studies a marked reduction in the zinc level in the serum and in the femur was noted in the animals given rapeseed protein concentrate (Jones, 1979; Ohlson and Anjou 1979; Shah *et al.*, 1980). Eklund and Agren (1974) reported that rapeseed protein concentrate caused adverse effects in pregnant rats, such as a loss of appetite, wasting, apathy, bleeding at the eyelids and nose, reduced litter size and an increase in numbers of stillborn pups. They suggested the presence of a toxic component other than glucosinolates in the rapeseed protein concentrate. However these symptoms are similar to those of zinc deficiency (Jones, 1979). Zinc supplementation of the protein concentrate diets elevated serum and femur zinc levels to those of the control rats (Jones, 1979). When the phytic acid content in the rapeseed material was reduced either by acid leaching, by using rapeseed with low phosphorus content, or by preparing a rapeseed isolate, no harmful effect was found (Ohlson and Anjou, 1979).

#### 2.2.6 Glucosinolates

The content of glucosinolates in rapeseed meal differs with variety, environmental and processing conditions. The difference in glucosinolate content in different varieties has been reported by many workers, e.g., Wetter and Craig, (1959); Appelqvist, (1962); Youngs *et al.*, (1972). Josefsson and Appelqvist (1968) studied the glucosinolate content in





different varieties. *Brassica napus*, winter type, has the highest glucosinolates content (6.11-6.40%), followed by *B. napus*, summer type, (4.31-5.03%), *B. campestris*, winter type, (3.32-3.44%), and *B. campestris*, summer type, (3.03-3.31%). The rapeseed varieties currently grown in Canada are of double and triple low varieties. For Candle, a triple low canola variety, the level of glucosinolates in the meal is between 1-2 mg/gm (Genser and Eskin, 1979).

According to Josefsson (1972) the glucosinolate content seems to be the main limiting factor in the use of rapeseed meal as feed. A nomenclature related to chemical structure by which the thioglucosides are all called glucosinolates was proposed by Ettlinger and Dateo (1961). All the 300 cruciferae that have been examined for the compounds contain glucosinolates (Ettlinger and Kjaer, 1968; Kjaer, 1960, 1963, 1966). To date there are 50 identified glucosinolates of different chemical structures. The structural characteristic of many of these compounds were identified by Kjaer and coworkers (Ettlinger and Kjaer, 1968). Although only one or two glucosinolates are usually present in a relatively large amount, as many as six of the compounds have been found in any given cruciferae species (Van Etten et al., 1969a). The structure of all glucosinolates is that of the prototype shown in Fig. 1 which was established by Ettlinger and Lundeen (1957).

Glucosinolates are hydrolyzed when the wet unheated plant material is crushed. The hydrolysis is catalyzed by an



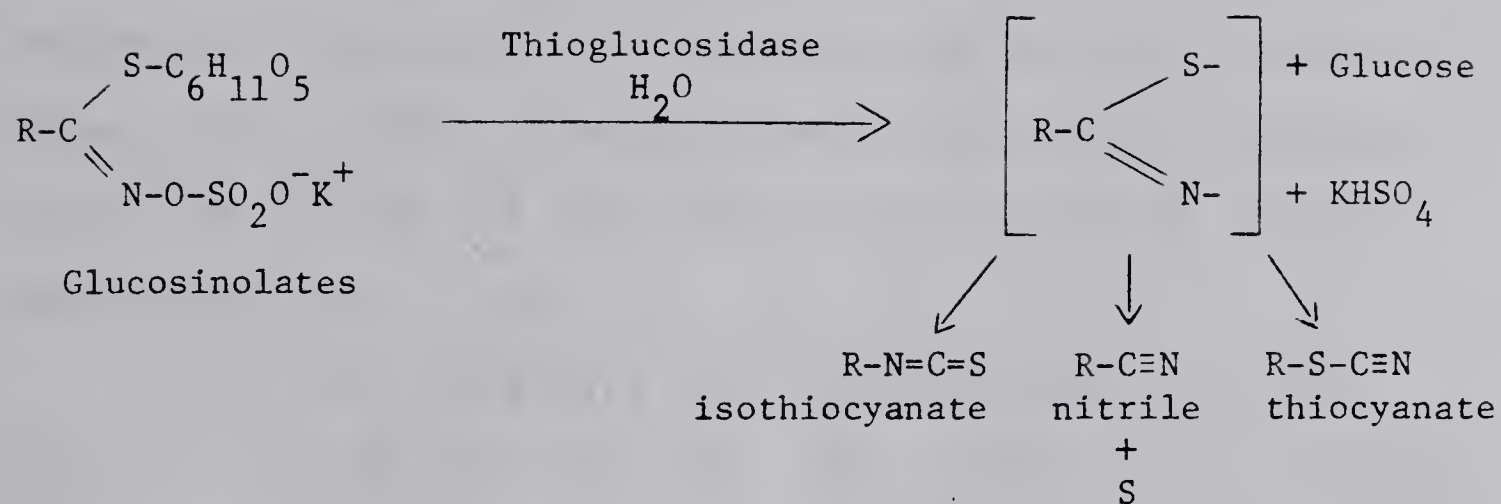


Fig. 1. Reaction Mechanism of Myrosinase (Thioglucosidase) on Glucosinolates (Ettlinger and Lundeen, 1957).



enzyme in the plant material (Fig 1) (Van Etten *et al.*, 1969a). In the early literature this enzyme was called myrosinase or mustard myrosinase. However, the International Union of Biochemistry recommends that the name thioglucosidase be used for an enzyme that hydrolyzes a thioglucosyl bond (VanEtten *et al.*, 1969a) Hydrolysis proceeds rapidly above a moisture content of 13% and at a temperature from 40-72°C. At a moisture level of 15.5% and temperature of 55°C, the hydrolysis was over 90% complete within one minute and 99% complete after fifteen minutes (Mustakas *et al.*, 1962).

During the hydrolysis of a glucosinolate an intra molecular arrangement may occur and produce isothiocyanates or thiocyanates. Nitriles and sulfur may be formed with no change in carbon skeleton (Challenger, 1959; Kjaer, 1960). Glucose and acid sulfate ions are always formed from the hydrolysis. The formation of isothiocyanates, thiocyanates and nitriles depends on the conditions of hydrolysis and other unidentified variables (Miller, 1966; Van Etten *et al.*, 1966; Virtanen, 1965). Each of the characterized glucosinolates (Ettlinger and Kjaer, 1968) differs from the others only in the nature of the R group (Fig. 1).

An example is progoitrin which in hydrolysis may give (S)-5-vinyloxazolidine-2-thione, also called goitrin. Goitrin was isolated from rape and characterized by Astwood *et al.* (1949) using paper chromatography.





The sulfur-containing products released upon the hydrolysis of glucosinolates are said to be "goitrogenic" (Van Etten *et al.*, 1969a). These compounds are antithyroid agents (Greer, 1962a). They act by inhibiting the organic binding of iodine in the thyroid. Their goitrogenic effect is not prevented by the addition of iodine to the diet (Van Etten *et al.*, 1969a).

## 2.3 DETOXIFICATION OF RAPESEED

### 2.3.1 Enzyme Myrosinase Deactivation

Currently there is a limited use of rapeseed meal in human and animal diet because of the glucosinolates content. When the glucosinolates are hydrolysed by the enzyme myrosinase, goitrogenic, highly toxic and growth-inhibiting compounds are released (Bell, 1965; Van Etten *et al.*, 1966). If myrosinase were inactivated beforehand, the meal would presumably be nontoxic since unhydrolysed glucosinolates are not goitrogenic. However, this method is of limited value because of the possible presence of other sources of the enzyme. Myrosinase activity has been found in the gut contents of rats (Greer, 1962b). Bacterial species such as *Paracolobactrum* found in the normal flora of the rat guts have shown the ability to convert the glucosinolates to the toxic compounds (Oginsky *et al.*, 1965).





Several studies have been published on the effect of dry and wet heat on the enzyme glucosidase (Eapen *et al.*, 1968; Sosulski *et al.*, 1972; Appelqvist and Josefsson, 1967). Sjollemma (1900) was cited by Anjou *et al* (1978) as the first reference regarding myrosinase inactivation through wet treatment at 72°C or through dry heat treatment at 100-105°C. At a moisture level of about 4%, heat treatment of rapeseed at 90°C for 15 minutes caused only a slight loss of myrosinase activity. On the other hand, the same treatment at a 6-8% moisture level will almost completely deactivate the enzyme. Treatment of rapeseed at 80°C for 15 minutes gave a similar degree of deactivation at 10, 8 and 6% moisture levels, whereas seeds with only 3.6% moisture behave differently. It appears that deactivation is dependent on free water which is present at 6.4% but not at 3.6% (Appelqvist and Josefsson, 1967).

Wet treatment, by immersing the intact seeds in boiling water, was effective in destroying the enzyme. The oil produced had a lighter color and less free fatty acids (Eapen *et al.*, 1968). Steam blanching of seed for five minutes destroyed the enzyme. However, steam blanching resulted in darker colored oil. The stability of myrosinase upon heating was reported to be high under dry conditions. Meal heated for six hours at 135°C still showed some enzyme activity (Bell and Belzile, 1965). Some enzyme activity was noticed in meals heated overnight at 110°C (Bhatty and Sosulski, 1972). Microwave heating for 1.5 minutes was not



effective in destroying the enzyme, but 3 minutes of exposure destroyed the enzyme completely. However, microwave heat for as short a period as 1.5 minutes reduced the quality of the oil as well as the meal (Eapen *et al.*, 1968).

The optimum moisture content in the seed for microwave deactivation of the enzyme was found to be between 14% and 16%. The amount of microwave energy required to deactivate the enzyme decreased as the moisture content of the seeds increased from 10 to 20%. In crambe seeds the enzyme could not be deactivated at a 7% moisture level without burning the seeds (Medeiros, *et al.*, 1978).

### 2.3.2 Destruction of Harmful Factors in Rapeseed Meal Through Heat Treatment or Reaction with Other Compounds

Several detoxification procedures have been reported in the literature. Steaming or toasting of the meal are the two most widely used treatments (Bell and Belzile, 1965; Rutkowski, 1970; Srivastava and Hill, 1976). The optimum nutritional value of detoxified rapeseed meal as evaluated by feed experiments with mice appears to be obtained when the seed is heat treated at 100-110°C for 15-60 minutes (Josefsson, 1975). Heat treatment may have an effect on high-molecular compounds in the meal. It destroys the factors causing formation of nitriles from glucosinolates even when meals are heated dry and the myrosinase remains active (Van Etten *et al.*, 1966; Josefsson, 1974). This may be a denaturation of specific, toxic, high molecular compounds





that possibly exist in rapeseed meal. This possibility should be considered for low as well as high glucosinolates meal; and there may also be less specific positive effects due to generally better digestibility of denatured protein (Josefsson and Munk, 1972). Goitrogenic agents are inactivated probably due to transformation of 5 vinyl-1.3 oxazolidine-2-thione to harmless 5 vinyl 1.3 thiozolidinone (Kozłowska *et al.*, 1974). Unfortunately, reduction of available lysine (Josefsson, 1975), extensive denaturation of protein, formation of Maillard type compounds and incomplete removal of goitrogenic compounds makes this method ineffective (Kozłowska *et al.*, 1974).

Ammoniation of glucosinolates to thiourea derivatives was only explored with *Crambe Abyssinica*, a crucifer related to rapeseed. The treatment gave crambe meal a significantly better nutritive value (Van Etten *et al.*, 1969a). Feeding experiments with chicks and cattle show improved palatability and nutritional quality (Kirk *et al.*, 1966). However, this process has not been widely accepted (Rutkowski, 1970). The degradation of glucosinolates for the release and removal of steam volatile nitriles has been accomplished by treatment with copper and iron salts (Youngs, 1967; Austin *et al.*, 1968). However, a decomposition product of oxazolidinethione, a hydroxynitrile (1-cyano-2-hydroxy-3-butene), which is toxic to animals, was only slightly steam volatile and remained in the meals.





Diluted sulfuric acid was found to degrade glucosinolates (Szewczuk *et al.*, 1969). However, the process resulted in considerable degradation of lysine.

Treating rapeseed flour with heat and using sodium metasilicate as a catalyst eliminates glucosinolates (Lanzani *et al.*, 1974).

Treatment with  $H_2O_2$  lowered the glucosinolates content of the flour but was not as effective as water extraction (Anderson *et al.*, 1975). Also the  $H_2O_2$  treatment caused oxidation of methionine to its sulfoxide and sulfone, and cysteine to cysteic acid.

### 2.3.3 Extraction of Glucosinolates and Hydrolysis Products

Glucosinolates and the products of their hydrolysis are soluble in water which makes a simple water washing technique to detoxify rapeseed meal possible. This approach seems to be the most economical and acceptable for detoxification (Tape *et al.*, 1970; Ballester *et al.*, 1973; Eklund *et al.*, 1971; Owen *et al.*, 1971). Glucosinolates are more soluble in water than isothiocyanates or oxazolidinethione (Belzile and Bell, 1966).

The extraction of glucosinolates from the meal by water was applied with varying degrees of success (Bell, 1957; Allen and Dow, 1952; Ballester *et al.*, 1970b, 1973; Kozłowska *et al.*, 1972a; Eklund and Agren, 1974; Mustakas *et al.*, 1976).



Tape *et al.* (1970) described a method for the removal of the glucosinolates from crushed rapeseed by aqueous extraction. The procedure consists of two thirty minute extractions at constant temperature each followed by a filter wash in a vibrator screen. Solid losses are considered excessive, although they have been reduced to 17%. Ballester *et al.*, (1970b) studied the effect of different treatments on the removal of the toxic factors in rapeseed. They varied from direct and indirect treatments to one and two step water extractions at different temperatures. Double extraction gave the best results by removing 84% of oxazolidinethiones (OTH) and 77% of isothiocyanates (ITC). Different treatments showed a slight increase in the meal digestibility. The first treatment used by Ballester *et al.* (1970b) took fourteen hours. They later proposed a new improved method (Ballester *et al.*, 1973) which is based on a continuous two-hour water extraction. They achieved a complete removal of ITC and a 97% reduction of OTH. The washed RSM caused a slight increase in the size of a rat's liver whose histology continued to be normal. Further studies on the washed meal (Ballester *et al.*, 1977) failed to clarify whether ITC and OTH are the only toxic substances present in rapeseed meal. It is possible that other toxic compounds present are also capable of inducing histological changes of a magnitude similar to those reported on the rat's thyroid. Various extracting solvents have been used, such as isopropanol ethanol, acetone ether,





buffer solutions, and dilute NaOH. Using solvents and their aqueous mixtures, different amounts of glucosinolates were extracted by different investigators (Bell, 1975; Tookey *et al.*, 1965; Afzalpurkar *et al.*, 1974; Mukherjee, 1975). In recent years new methods of detoxification have been reported for whole or crushed rapeseed (Bhatty and Sosulski, 1972; Eapen *et al.*, 1968; Sosulski *et al.*, 1972; Tape *et al.*, 1970). The detoxification of whole seeds by "diffusion extraction" was done by the extraction of whole seeds in water (Sosulski *et al.*, 1972; Kozłowska and Sosulski, 1972a), or in an aqueous solution of ethyl alcohol (Bhatty and Sosulski, 1972; Kozłowska and Sosulski, 1972b). Four two-hour extractions or six one-hour extractions were most efficient for the diffusion of glucosinolates with minimum losses of other seed constituents. Loss of soluble solids was about 15%, loss of nitrogen did not exceed 10%, and most of the nitrogen diffused from the seed was nonprotein nitrogen. The principal disadvantages of the ethanolic sodium hydroxide procedure were reduced solubility of the rapeseed protein and the higher fibre level of the meal (Bhatty and Sosulski, 1972). Boiling the seed before diffusion extraction or the use of high temperature or highly alkaline solvents were effective in controlling the myrosinase activity and sulfur level in oil (Sosulski *et al.*, 1972; Kozłowska *et al.*, 1972a; 1974; Sosulski, 1974).

A new two-stage process was developed by Woyewoda *et al.* (1978). This method entails preliminary hydrolysis of





glucosinolates and subsequent removal of glucosinolates aglycones by activated carbon treatment at pH 10. The result was 99.96% elimination of aglycones of glucosinolates present in the original solution. This method removed glucosinolates from their pure aqueous solution when hydrolyzed. However, the results could not be duplicated with a solution containing rapeseed protein.

The use of ultrafiltration in separating glucosinolates (molecular weight 400) from proteins (molecular weight 15,000) was studied at Karlshamn oil factories in Sweden. This method removed 93% of the glucosinolates (von Bockelmann *et al.*, 1974).

#### 2.3.4 Biological Detoxification

Staron (1970, 1974) developed a method to eliminate the toxic suflur compounds through fermentation. Fifty very diverse micro-organisms (bacteria, yeasts, fungi) were tried, six of which gave satisfactory results. He selected *Geotrichum Candidum* (a yeast) which degraded glucosinolates in rapeseed meal after 86 hours incubation at 37°C. Lactic acid bacteria provided similar results (Kozłowska *et al.*, 1972b).

#### 2.3.5 Removal of Glucosinolates by Plant Breeding

According to Kondra (1977) "plant breeding technique is the most satisfactory method for eliminating or significantly reducing the glucosinolates". Josefsson *et*



al.(1975). suggested that plant breeding research should be continued until glucosinolates are completely removed.

A successful rapeseed variety must possess an appropriate combination of characteristics important both to the agronomist and to the industrial processor. The important agronomic characteristics include high-yielding ability, early maturity, as well as resistance to shattering, lodging, and to certain diseases. For the industrial processor the quality of the mature seed is of importance. Seed quality should include bigger seeds with higher oil and higher protein content. The oil must conform to a certain pattern of fatty acid composition and the seed should be free from minor undesirable constituents such as the glucosinolates. It should be low in fibre, attractive in appearance and rich in essential amino acids (Appelqvist, 1972a). Canadian research in this field has been successful in producing varieties with a chemical make-up suited to specific uses. The rapeseed varieties currently grown in Canada are of the *Brassica napus* species (Polish type) and *Brassica campestris* species (Argentine type) (Kondra, 1977).

Oil from rapeseed grown in Canada prior to 1970 contained from 22-45% erucic acid depending on the variety. Erucic acid compromised the nutritional value of the oil while goitrogenic glucosinolates limited the feeding value of the meal. Canadian varieties have been genetically modified to correct these problems. The Canadian crop is gradually being converted to the low erucic acid and low





glucosinolates (Kondra, 1977; Genser and Eskin, 1979). Candle, the first strain of *Brassica campestris* low in both erucic acid and glucosinolates, was introduced in Canada in 1976. This yellow seeded canola variety, referred to as triple low, is also low in fibre (Genser and Eskin, 1979; Kondra, 1977, 1979). The Candle has oil which is low in erucic acid and meal which contains no more than 2 mg of glucosinolates expressed as 3-butenyl isothiocyanate per gram of moisture free and oil free meal (Genser and Eskin, 1979).

#### 2.4 RAPESEED PROCESSING

The processing of rapeseed to obtain oil and meal is similar to the methods employed for other high oil content seeds. Three types of processing are currently employed in Canada: expeller processing, prepress solvent extraction, and straight solvent extraction. Prepress solvent extraction is the most widely used (Youngs *et al.*, 1978). These processes produce the oil as the main product and the meal as the by-product.

The glucosinolates content limits the use of the meal in both animal and human diet (Bell, 1965). Detoxification is desirable to improve the nutritional value of the meal. In recent years several methods have been developed to detoxify the meal as was discussed earlier. New techniques have been developed lately. Some of these techniques process





rapeseed to oil and nontoxic meal or protein concentrate. Other techniques utilize the meal for the production of nontoxic protein concentrate. These new techniques include: the extraction by solvent of the detoxified, dehulled seeds (Anjou and Fecske, 1974; Anjou *et al.*, 1978; Jones, 1979; Ohlson, 1973; Kozłowska *et al.*, 1974); the aqueous extraction of the seed to wash out most of the oil, glucosinolates and soluble carbohydrates (Embong and Jelen, 1977); the hydraulic pressing of dehulled seed (Agren and Eklund, 1972); and the extraction of protein concentrate from meal by saline extraction (Lo and Hill, 1971; Owen *et al.*, 1971); or alkaline extraction (Sosulski and Bakal, 1969, Girault, 1973, El-Nockrashy *et al.*, 1977, Yang *et al.*, 1978). Embong (1977) compiled an extensive review of these techniques.

#### 2.4.1 Expeller Processing

This is a mechanical process in which the oil is squeezed from the seed. The seed must be dried to 6.9% moisture content to avoid any myrosinase activity in the crushed seeds. The heated rapeseed is then crushed to facilitate the subsequent cooking and extraction. Crushing breaks the seed structure allowing the separation of oil and meal. Crushing is done using one or a few pairs of rollers which may be smooth or corrugated. Crushing should not be too intense in order to avoid producing large amounts of fines which may cause difficulties during the extraction



process. Before pressing and extraction, the crushed seed is subjected to a heat treatment which is called conditioning or cooking (Youngs *et al.*, 1978; Anjou, 1972). The main objectives of cooking are: completion of the breakdown of oil cells; coagulation of the protein to facilitate the separation of the oil, reduction of the affinity of the oil to the solid surfaces, insolubilization of phosphatides and increased fluidity of the oil by an increase in temperature (Swern, 1964). Cooking also deactivates lipase resulting in a lower free fatty acid value and hence lower refining costs. Cooking increases the quality of the oil cake by the destruction of myrosinase, lipoxidase, mold and bacteria. Cooking is usually applied for 30-60 minutes at a temperature varying from 75-120°C, using stack cookers or continuous horizontal cookers (Anjou, 1972). The crushed, cooked seed then passes to the expeller or screw press. During pressing, an extremely high pressure of 1000-1400 kg/cm is applied producing a temperature of 150°C. The oil content of the cake can be reduced to 4% during the pressing, but it may be necessary to leave 6-7% to avoid damage to the cake. The cake coming straight from the expeller is both hot and dry, and water may be added at this point to reduce the temperature and increase the moisture content. The cake is then ground into meal ready to be used in animal feeds (Youngs *et al.*, 1978; Anjou, 1972).



#### 2.4.2 Prepress Solvent Extraction

In this process a portion of the oil is removed from the cleaned seed by expellers and the remaining oil is then extracted with an organic solvent. The pretreatment of the seed and the expeller used for processing are the same as those described in the previous section. In this case, however, only 70-80% of the oil is removed by pressing. This requires much less pressure than in the straight expeller process where oil recoveries of 90% are desired. The cake from the expellers, containing 15-20% oil, is extracted with commercial hexane in a continuous counter-current extraction to remove as much oil as possible. The solvent in the meal is stripped in desolventizers. The meal obtained contains about 2% fat, with moisture content of about 10% (Anjou, 1972; Youngs *et al.*, 1978).

#### 2.4.3 Direct Solvent Extraction

Normally, seeds with high oil content such as rapeseed are not directly solvent extracted as they tend to disintegrate into fine particles when placed in solvent, which makes counter-current movement of the meal impractical. A process known as 'filtration-extraction' (D'Aquin *et al.*, 1953) has been developed which overcomes this problem and it has been successfully applied to rapeseed processing.







#### 2.4.4 Solvent Extraction of Dehulled Detoxified Seeds

The conventional methods used currently in rapeseed processing included a treatment to destroy the enzyme myrosinase, however, the potentially harmful glucosinolates stay with the meal. Because of these substances, rapeseed is not being used as a source of protein concentrates or protein isolate for human consumption (Kozłowska *et al.*, 1974). In recent years, new methods of detoxification were reported for whole and crushed rapeseed (Bhatty and Sosulski, 1972; Eapen *et al.*, 1968; Sosulski *et al.*, 1972; Tape *et al.*, 1970). These methods created new possibilities for the use of rapeseed protein for human nutrition by removing all the glucosinolates while preserving the protein (Kozłowska *et al.*, 1974).

Two Swedish companies (A.B. Karlshamns Oljefabriker and Alfa-Laval A.B.), have together developed a new process for the production of a detoxified, bland, light-colored protein concentrate from rapeseed, which has a very high nutritional value (Anjou and Fecske, 1974). Similar methods were described by Kozłowska *et al.* (1974), Jones (1979), Ohlson (1973), Anjou *et al.* (1978). In general, there are four major steps in the preparation of rapeseed protein concentrate: inactivation of myrosinase; removal of glucosinolates; dehulling; and oil extraction. These steps may be combined in as many as 6 different process orders (Anjou and Fecske, 1974). The myrosinase is inactivated in boiling water. This step also reduces the microorganism



population in the seeds and lowers the protein solubility (Anjou and Fecske, 1974; Anjou *et al.*, 1978; Jones, 1979). The glucosinolates are removed by diffusion extraction which depends on the extraction of whole seeds in water (Sosulski *et al.*, 1972; Kozłowska and Sosulski, 1972a,b; Kozłowska *et al.*, 1974); or by water leaching of the cracked seeds (Anjou and Fecske, 1974; Anjou *et al.*, 1978). These processes remove glucosinolates, soluble carbohydrates, especially problem sugars such as raffinose and stachyose, and phenol-like compounds such as sinapine. The diffusion extraction is enhanced by frequent changes of solvent, high temperature, and the neutral to alkaline pH of the aqueous medium (Sosulski *et al.*, 1972). A sodium hydroxide solution of 0.01 N at 60°C was used for the diffusion extraction. The intact seeds needed more than three hours, while 1.5 hours of extraction was enough in the case of dehulled seeds (Kozłowska *et al.*, 1974). Leaching extraction needed a total time of 5 hours (Anjou *et al.*, 1978). Dehulling was done either before or after detoxification. It is recommended to remove the hulls before detoxification to reduce the volume of solvent used (Anjou and Fecske, 1974; Kozłowska *et al.*, 1974). The dehulled detoxified seed is hexane-extracted to produce oil and protein concentrate. Yields of products from the rapeseed protein concentrate process were 12-15% hull fraction, 3-10% fines, 13-16% dry matter from evaporated leaching water, 35-42% oil and 23-28% rapeseed protein concentrate (Anjou and Fecske, 1974). This protein





concentrate contained 67% protein, 7% fibre, 7% ash, and less than 0.1% glucosinolates (Ohlson and Tear, 1974).

#### 2.4.5 Hydraulic Pressing of Dehulled Seed

Agren and Eklund (1972) described a method for the hydraulic pressing of dehulled rapeseeds. The process resulted in a meal with comparatively low oil and crude fibre content. Using this process, about 93% of the oil in the dehulled seeds was extracted. The hydraulic pressing is carried out at room temperature. Repeated washing of the resultant meal with water is required to remove glucosinolates.

#### 2.4.6 Aqueous Extraction

Oil and protein can be recovered simultaneously from oilseed materials by a process which consists of an aqueous extraction of the comminuted seed, followed by a centrifugal separation of the slurry into oil, solid and aqueous phases. The aqueous extraction of oleaginous materials is not new. It has been used in the Orient with coconut for a long time (Hagenmaier, 1977). Earlier studies have been reported on aqueous extraction, but with no commercial use (e.g., Van Deurs, 1928; Hocker, 1930; Ludecke, 1938; Nyrop, 1938). During the last two decades, several new processes for the aqueous extraction of oil and protein from oil seeds have been suggested (e.g., Sugarman, 1956; Rhee *et al.*, 1972, 1973; Hagenmaier *et al.*, 1972, 1973; Hagenmaier, 1974;





Hagenmaier, 1977; Cater *et al.*, 1974; Mieth *et al.*, 1975a, Embong and Jelen, 1977). Some of the advantages of aqueous extraction over conventional solvent extraction are: the elimination of flammable hexane, which means safer operation; no air pollution from solvent losses; less initial capital investment in machinery, and the production of good quality oil and protein in fewer steps (Embong, 1977; Cater *et al.*, 1974). The disadvantages of aqueous extraction include lower efficiency of oil extraction. At best the process yields only 95% of the oil compared to the conventional methods. This results in high oil residues in the meal which means an economical loss. Also, it may cause an off flavour and hence a storage stability problem, with an increased potential for microbial contamination (Embong, 1977; Cater *et al.*, 1974).

Embong (1977) reviewed the aqueous extraction process in detail. He also described a method for the aqueous extraction of rapeseed, and reported the extraction of over 90% of the oil. The quality of the aqueous extracted oil was better than industrial crude rapeseed oil. It contained lower amounts of phospholipids, sulfur and free fatty acids.

The efficiency of the process is critically dependent upon several unit operations: grinding, extraction, centrifugation, breaking the emulsion and drying (Embong, 1977; Cater *et al.*, 1974; Hagenmaier *et al.*, 1972). The following is a general description of each unit operation:



(a) *Grinding* is critically important. Fine seed cells must be ruptured to release their constituents and increase efficiency of extraction (Cater *et al.*, 1974). Insufficient grinding results in unacceptable losses of oil in residues (Embong, 1977; Cater *et al.*, 1974; Rhee *et al.*, 1972). Excessive grinding can produce smaller oil globules which makes breaking the emulsion more difficult (Cater *et al.*, 1974; Hagenmaier *et al.*, 1972). Grinding can be carried out with either wet or dry seed, depending upon the initial moisture content, chemical composition and physical structure of the oilseeds (Cater *et al.*, 1974; Embong, 1977).

(b) *Extraction*: Basically, the extraction step is carried out by dispersing the ground seeds in water and then agitating the dispersion to enhance the extraction of seed constituents. Factors which influence the efficiency of extraction include: solid to water ratio; pH of the dispersion; extraction time and temperature, and the degree of agitation (Cater *et al.*, 1974; Embong, 1977; Hagenmaier, 1977).

(c) *Solid-Liquid Separation and Centrifugation*: After extraction and before centrifugation, the removal of solids such as fibrous material, undissolved carbohydrates and protein has been found to be necessary with some oilseeds. The removal could be accomplished by a clarifying centrifuge or any other method, depending on the nature of the material and the cost (Cater *et al.*, 1974; Embong, 1977). This is





followed by a three-phase centrifugation in which the liquid containing both dissolved and undissolved materials is separated into oil, solid and aqueous phases. Oil can be recovered as free oil or oil-in-water emulsion, depending on the processing conditions (Embong, 1977; Carter *et al.*, 1974).

(d) *Breaking the Emulsion*: If an emulsion has been formed, it can be broken using different techniques such as the phase-inversion technique described by Sugarman (1956), or heating and centrifugation (Rajasekharan and Sreenivasan, 1967), low temperature (freezing and thawing) (Roxas, 1963; Embong and Jelen, 1977), and centrifugation which was reported to break the coconut oil emulsion completely (Hagenmaier *et al.*, 1972; Dendy and Timmins, 1974).

(e) *Drying*: The removal of water from protein products is usually achieved by spray drying (Cater *et al.*, 1974).

#### 2.4.7 Extraction of Protein From Rapeseed Meal Using Sodium Chloride

Bhatty *et al.* (1968) indicated that 67% of rapeseed protein was soluble in 10% sodium chloride solution. This suggested that it might be possible to prepare protein concentrate from rapeseed using a saline extraction procedure. Using this technique Owen *et al.* (1971) produced a very pure protein; the yield was 18% of the total nitrogen. Lo and Hill (1971) proposed a procedure for preparing protein concentrate that yielded 75% of the





original nitrogen of the starting meal. The product contained 61-64% protein, a high content of ash, and less crude fibre and glucosinolate than the original meal.

Further work (Girault, 1973) indicated that higher yields could be obtained using the alkaline extraction than by using the saline extraction.

#### 2.4.8 Alkali Extraction

Alkali treatment of foods and food proteins has been used since ancient times. For example, Central American Indians have treated corn with alkali for many generations (Katz *et al.*, 1974). This treatment, which makes vitamin niacin more nutritionally available, may be important in controlling pellagra in these areas (Finley *et al.*, 1977).

Exposure of protein to alkali is increasingly applied in technological treatment of food and feeds, e.g., for dissolving proteins in the preparation of concentrates and isolates (Sullivan, 1943; Sosulski and Bakal, 1969; Rutkowski and Korolczuk, 1974); for obtaining proteins with specific properties such as foaming, emulsifying or stabilizing (Circle and Johnson, 1958); for the preparation of textured vegetable protein (Hamdy, 1974); for the processing of fruits and vegetables (Hart *et al.*, 1974); and for the destruction of aflatoxin in peanuts (Screenwasamurthy, 1967).

Several investigators have described techniques for solubilization and extraction of crude protein preparation



from rapeseed meal and flour. The techniques involve pH manipulation, either in one step by dispersing the raw material in an aqueous alkaline solution and then recovering the dissolved protein by acid precipitation after the removal of the insoluble materials; or in a multiple step extraction process which under industrial conditions makes a more complete recovery of the protein from the raw material possible (Gillberg and Tornell, 1976; Kodagoda *et al.*, 1973a, Girault, 1973, Sosulski and Bakal, 1969, Pokorny and Sefr, 1964, El-Nockrashy *et al.*, 1977, Yang *et al.*, 1978).

Rutkowski and Korolczuk (1974) suggested that the extraction can be done sequentially at different pH conditions. They found that the highest yield of protein can be obtained from rapeseed meal by a three stage extraction following the system water-sodium hydroxide-water.

All the above scientists worked either in rapeseed meal or flour. No work seems to have been done on the alkaline extraction of full fat rapeseed. However, reports are available on the alkaline extraction of protein and oil from sunflower (Hagenmaier, 1974), peanut (Rhee *et al.*, 1973), and cottonseed, tung nut, peanut and soybean (Sugarman, 1956). The processes consist of grinding oilseeds in an alkaline solution to produce a slurry which is diluted and centrifuged to produce solid, concentrated oil emulsion and clarified protein solution. The protein is acid precipitated, filtered, washed and dried. The oil is extracted from the emulsion by exerting shear and pressure





forces at the emulsion at a certain pH thus breaking the emulsion. Centrifugation is applied to remove the oil from the broken emulsion. The above process produces pure oil, protein and meal. Embong (1977) did not explore the alkaline extraction in his work with rapeseed; he was concerned primarily with maximizing oil extraction at a neutral pH.

Hagenmaier (1974) measured the free fatty acids of oil of sunflower seeds which were incubated in water for one hour with 0.2%  $\text{Na}_2\text{SO}_3$  added. No change in free fatty acid was observed over the range 5-65°C and pH 4.5-10. In other studies with peanut, Rhee *et al.* (1973) found that free fatty acid value increased slightly with increasing extraction pH.

## 2.5 ALKALINE EXTRACTION OF RAPESEED PROTEIN

### 2.5.1 Effect of pH on Protein Solubility

The most important factor controlling the alkaline extraction of rapeseed protein is pH (Rutkowski and Korolczuk, 1974). The solubility profile of different varieties of rapeseed was studied by several investigators (Quinn and Jones, 1976; Radwan and Lu, 1976; Korolczuk and Rutkowski, 1971; Rutkowski and Korolczuk, 1974; Gillberg and Tornell, 1976; and Yang *et al.*, 1978). The nitrogen solubility curves have a rather complex shape. This can be attributed, at least partly, to the fact that rapeseed has a





very complicated protein system and contains proteins with isoelectric points in the pH range 4-11 (Gillberg and Tornell, 1976). The rapeseed meal shows high nitrogen solubility, even at the solubility minimum at about pH 4 (Gillberg and Tornell, 1976).

The minimum point of nitrogen extractability was found to occur at pH values of 3.7-4 when extraction was conducted at 27°C (Quinn and Jones, 1976). Other reported values were slightly above pH 4 (Korolczuk and Rutkowski, 1971, Rutkowski and Korolczuk, 1974), pH 4.5 at 25°C (Radwan and Lu, 1976), and above pH 5 (Yang *et al.*, 1978). Soybean minimal values were reported between 3.5-4.5 at room temperature (Smith *et al.*, 1959). The reported values in the work of Sosulski and Bakal (1969) for rapeseed, flax and sunflower were in the range of 4.4-4.6. Maximum solubility was reported to increase at extreme pH conditions, with the peak at the alkaline side (Radwan and Lu, 1976; Gillberg and Tornell, 1976), at pH 11 (Yang *et al.*, 1978), and at pH 9.6-9.7 (Rutkowski and Korolczuk, 1974).

The rapeseed meal exerts some buffering action, particularly at pH values around minimum solubility (Radwan and Lu, 1976). Rapeseed protein behaves differently than other well-known oilseed proteins such as soybeans, peanuts and sunflower. Rapeseed has high proportion of its protein soluble over the acidic pH range (Mattil, 1971, Gillberg and Tornell, 1976). This solubility presents difficulties in the production of enriched protein products, which may indicate



a possible functional role in acidic food products that other proteins might not fulfill because of their low solubility at acidic pH (Quinn and Jones, 1976).

#### 2.5.2 Effect of Pre-Extraction Heat Treatment on Nitrogen Solubility

In order to deactivate the myrosinase, the seeds are subjected to heat treatment (Appelqvist and Josefsson, 1967). Such a heat treatment may decrease the solubility of the protein and hence have a negative effect on the yield of the protein extraction (Girault, 1973). Dry heat has a negligible effect on protein solubility with or without the presence of oil. On the other hand, wet-heat even if it is limited, leads with time and temperature to progressive insolubilisation (Girault, 1973; Gillberg and Tornell, 1976). Rutkowski (1970) reviewed the effects of industrial treatments on the chemical composition of the meal. He demonstrated the unfavorable effect of temperature in humid environment on protein solubility and browning reaction. Gillberg and Tornell (1976) studied the effects of different heat treatments of the seeds on the properties of the defatted dehulled seeds. They indicated that the decrease in the nitrogen solubility produced by a heat treatment of the seeds, could be overcome to a certain extent by working the alkaline extraction at a high temperature. They also suggested that a technical process for the preparation of rapeseed protein isolate has to be based on a protein





extraction step conducted at a fairly high pH. This is especially true if the seeds to be processed have been subjected to a heat treatment.

### 2.5.3 Effect of Temperature on Protein Extractability

The minimum solubility of rapeseed protein increases with temperature. The pH value corresponding to these minimum values also increases with temperature (Radwan and Lu, 1976). The minimum solubilities of protein of Tower rapeseed in aqueous solution were 14.2%, 19%, 26.2% and 30.2%, at temperatures of 25°C, 35°C, 45°C, and 55°C. The corresponding pH values were 4.5, 4.8, 7, and 7.2, respectively (Radwan and Lu, 1976). These results also indicated that at pH 11 the percent of extracted nitrogen increased with the increase in temperature. Percentages of extracted nitrogen were 28, 34, 43 and 50% at temperatures of 25, 35, 45, 55° respectively.

Rutkowski and Korolczuk (1974) reported that as far as the alkali extraction is concerned, an increase in protein solubility occurs up to a temperature of 45°C. At pH 7 increasing the extraction temperature from 12 to 40°C caused an increase of 3% in extraction yield that followed with a decline in the yield at temperatures above 40°C (Thompson *et al.*, 1976). This can be attributed to the denaturation of the more heat sensitive rapeseed proteins (Shemer *et al.*, 1973). The increase in extraction with the increase in temperature is due to changes in the dissociation constant





of the protein with temperature. A higher temperature allows the hydrophobic amino acids in the interior of the protein to become exposed to the solution. In this new configuration they are thus able to affect changes in the shape of the solubility curve (Radwan and Lu, 1976). The extraction temperatures under alkaline condition reported in the literature are: 20°C (Girault, 1973), 40°C (Kodagoda *et al.*, 1973a), 50°C at pH 2 in the first extraction and 40°C at pH 10 in the second extraction (Keshavarz *et al.*, 1977), 30°C (El-Nockrashy *et al.*, 1977; Rutkowski and Korolczuk, 1974), room temperature (Quinn and Jones, 1976), and 4°C (Yang *et al.*, 1978).

#### 2.5.4 Effect of Extraction Time

Different extraction times were reported by different workers. In a single step extraction, the times reported were 30 minutes (Yang *et al.*, 1978; Rutkowski and Korolczuk, 1974); 60 minutes (Radwan and Lu, 1976; Girault, 1973; Sosulski and Bakal, 1969); 90 minutes (El-Nockrashy *et al.*, 1977). With a multiple step extraction, the conditions used were three minutes for three times at the same or different pH and temperatures (Kodagoda *et al.*, 1973a), 10 and then 30 minutes at different pH (Thompson *et al.*, 1976), 10, 15 and 15 minutes at different pH and temperatures (Keshavarz *et al.*, 1977), and 4 steps each for 10 minutes (El-Nockrashy *et al.*, 1977; Yang *et al.*, 1978). Rutkowski and Korolczuk (1974) indicated that the most important role is that of pH.



Increases in yield were observed upon increasing extraction time up to 30 minutes; long time up to 4 hours did not increase the yield.

#### 2.5.5 Effect of Solvent to Solids Ratio

Generally, the volumes of solvent to solids ratios used are in the range of 10-20 ml solvent/gm of meal for single or multiple step extraction. The choice of the ratio depends on economic considerations. On one hand, the higher the solvent volume the higher the yield (Rulkowski and Korolczuk, 1974). On the other hand, higher volume of solvent will raise the cost of isolation and increase the consumption of water and energy. Pokorny and Seif (1964) studied the effect of extraction with 0.5% NaOH. They found the highest yield at 15 ml/gm meal but they recommended the use of 10 ml/gm meal from which they obtained the highest concentration of nitrogen in dry mass of extract.

El-Nockrashy *et al.* (1977) studied ratios ranging from 100:1 to 20:1. They recommended the use of 25:1 in a multiple step counter current extraction. This ratio was selected because the use of smaller volumes of solvent leads to difficulties in extraction in later steps as a result of the high retention of solvent by the meal at the first step.

A ratio of 20 ml solvent/gm of meal was used by Sosulski and Bakal (1969) with rapeseed, flax, sunflower and soybean. For rapeseed extraction ratios of 20:1 (Girault, 1973; Yang *et al.*, 1978), 10:1 (Keshavarz *et al.*, 1977) and





16:1 (Quinn and Jones, 1976) were reported.

#### 2.5.6 Alkaline Agent Used in Extraction

The use of NaOH solution of normalities ranging from 0.02N to 0.2N has been recommended by several investigators working on the isolation of proteins from rapeseed meal (Pokorny and Serf, 1964; Sosulski and Bakal, 1969; Girault, 1973; Kodagoda *et al.*, 1973a., Rutkowski and Korolczuk, 1974). El-Nockrashy *et al.* (1977) made a comparison between sodium carbonate and sodium bicarbonate buffer at pH 10.0 and 10.6, and sodium hydroxide solutions ranging from 0.02N to 0.05N. Sodium hydroxide was found to be the most suitable solvent.

#### 2.5.7 Precipitation and Yield of Extracted Proteins

The extracted soluble proteins are precipitated at the isoelectric point. There has been controversy regarding the pH at which the maximum yield of rapeseed protein could be obtained (Pokorny and Sefr, 1964; Sosulski and Bakal, 1969; Girault, 1973; Kodagoda *et al.*, 1973a; Rutkowski and Korolczuk, 1974; Finlayson, 1966; Owen, 1973). The highest yield of protein was obtained at pH 3.5-3.9 (Pokorny and Sefr, 1964; Korolczuk and Rutkowski, 1971; Rutkowski and Korolczuk, 1974), at pH 4.2 (Kodagoda *et al.*, 1973a; Keshavarz *et al.*, 1977), while 4.4-4.6 was used by Sosulski and Bakal (1969). Using the one step precipitation, the yield was rather low and much of the dissolved protein





remained in the supernatant (Gillberg and Tornell, 1976; Yang *et al.*, 1978). The reported maximum precipitation was 56% (Girault, 1973), 49% (Pokorny and Sefr, 1964), or 55% (Gillberg and Tornell, 1976) of the dissolved protein.

The low yield of precipitation is mainly due to the fact that the extract contains a large number of different proteins having widely different molecular weights and isoelectric points (Yang *et al.*, 1978; Gillberg and Tornell, 1976). The use of precipitation aids such as sodium alginate, sodium hexametaphosphate, and carboxy-methyl cellulose (CMC) was found to increase the yield considerably (Gillberg and Tornell, 1976; Yang *et al.*, 1978). A stepwise precipitation at more than one isoelectric point was also used. The result was an increase in the reported yield to 63% (Gillberg and Tornell, 1976), 73% (Yang *et al.*, 1978), and 93.7% (El-Nockrashy *et al.*, 1977). Recent investigations have indicated that rapeseed may contain two main groups of proteins, one of relatively high molecular weight which dissociates into subunits at acid pH values, and another of lower molecular weight (Bhatty *et al.*, 1968; Finlayson *et al.*, 1969; Janson, 1971; Coding *et al.*, 1972; Girault, 1973). A process for isolation of protein based on these findings has not been developed (El-Nockrashy *et al.*, 1977).

All the above work was related to defatted meal or flour. As indicated before, no work seems to have been done on the full fat rapeseed. Sugarman (1956) patented a process based on the alkaline extraction of oilseeds in general.



### 2.5.8 Effect of Alkaline Extraction on Rapeseed Protein Color

The color of rapeseed protein varies from light yellow to a greyish-yellow-brown (Ohlson and Tear 1974). The usefulness of a product in wide range of prepared foods depends on its color (Smith, 1958). A dark brownish or greenish color which usually develops in protein extracted from rapeseed meal, particularly under alkaline conditions, has been a concern of researchers investigating potential utilization of these proteins in food processing (Rutkowski and Korolczuk, 1974; Radwan and Lu, 1976; Thompson *et al.*, 1976; Sosulski and Bakal, 1969; Yang *et al.*, 1978). During the alkaline treatment of rapeseed meal, the color of the meal changes gradually from yellow to grey in the direction of increasing acidity and from yellow to dark green in the direction of increasing alkalinity. The behaviour is more pronounced in the extract. No change in the meal color was noticed at or near minimum solubility points (Radwan and Lu, 1976).

The precipitation of alkaline protein extracts (prepared at pH 11.1) produced protein with different colors at different pHs (Yang *et al.*, 1978). Precipitation at pH 6.7 results in the formation of grey curd; at pH 5.6 the precipitate has a light cream color and at pH 5 a yellow cream color.

The same alkaline treatment of rapeseed, sunflower, flax and soybean produced dark brown and green isolates from





rapeseed and sunflower respectively, a tan color in flax protein and a creamy white color in soybean (Sosulski and Bakal, 1969). The dark color seriously limits the potential uses of rapeseed protein; for instance, when more than 6% of the wheat flour in a bread dough was substituted with a rapeseed protein, the color of the bread darkened (Kodagoda *et al.*, 1973b). The tan color of flax protein makes it more attractive to be added to dark-colored foods; on the other hand, the creamy white color of soybean extract makes it easy to incorporate it to a wide range of food without any observable effect on their appearance (Sosulski and Bakal, 1969).

Smith (1958) reported that the dark green-to-brown appearance of sunflower protein was due to the formation of an insoluble compound between polyphenols (chlorogenic acid) and the isolated proteins. Lo and Hill (1972) separated caffeic acid and chlorogenic acid from rapeseed protein products. It is possible that the protein-polyphenol interaction products are involved in the formation of the green and brown color of rapeseed protein products (Keshavarz *et al.*, 1977; Rutkowski and Kozłowska, 1979).

Another possible cause for the dark color is the formation of melanophospholipid as a result of hydrolysis and oxidation of phospholipids during the oil extraction from rapeseed (Aref, 1970). Appelqvist (1971) suggested that the seed coat pigments were responsible for the undesirable dark color of meal and of the extracted proteins from





rapeseed. The brown pigment of the isolate was not soluble in either polar or nonpolar organic solvents and could not be removed by gel filtration (Rutkowski and Korolczuk, 1974). Attempts to remove the pigments from the isolates by repeatedly dissolving the protein in an alkali solution and coagulation at pH 3.8, failed to give positive results. It even caused further darkening of the protein isolates (Rutkowski and Korolczuk, 1974).

The color of protein isolates can be considerably improved by washing twice; first, by water using a sludge-to-water mix ratio of 1:2, and second, by dry acetone (Yang *et al.*, 1978). The extraction of the meal using water as a first step improved the final alkaline extract (Rutkowski and Korolczuk, 1974). Additional washing of the isolates with polar organic solvents such as 50% methanol, ethanol, isopropanol and acetone, led to the removal of the traces of flavours and counteracted the browning during drying (Rutkowski and Korolczuk, 1974). Gheyasuddin *et al.* (1970) reported that the addition of sodium sulfite was effective in obtaining colorless protein isolate from sunflower seed; however, Hagenmaier (1974) was not able to duplicate the results.

#### 2.5.9 Chemical and Nutritional Modification of Proteins due to Alkaline Processing

The ability of strong alkali to racemize amino acids was reported by several investigators (Provansal *et al.*,



1975; Tannenbaum *et al.*, 1970; Masters and Friedman, 1979). The reaction is thought to proceed by the withdrawing of a proton from an amino acid or an amino acid residue to give a negative charged planar carbanion. A proton can then be added back to either side of this active intermediate, thus regenerating the L form or producing the D enantiomer (Masters and Friedman, 1979). Racemization was found to occur in the amino acids phenylalanine, serine, cystine, methionine, and tyrosine (Hill and Leach, 1964; Pollock and Frommhagen, 1968). Severe treatment with sodium hydroxide (0.2 M, 80°C, one hour) caused a marked degree of isomerization of the L-lysine residues to D-lysine (Provansal *et al.*, 1975). Tannenbaum *et al.* (1970) found methionine to be almost completely racemized in fish protein concentrate heated for 20 minutes at 95°C in 0.2N NaOH.

Racemization rates of individual amino acids may vary among proteins. A processing condition which may be mild for one protein (e.g., lactoalbumin), could be rather severe for another protein (e.g., promine-D). However, in any given protein, the relative rates are similar (Masters and Friedman, 1979). Racemization can impair nutritional values of food proteins by decreasing the amounts of the essential L amino acids present, by decreasing digestibility, and as a result of specific toxicity of certain D enantiomers (Provansal *et al.*, 1975; Masters and Friedman, 1979).

It is well known that severe alkaline treatments can cause chemical modification and destruction of amino acid





residues such as cystine, arginine, thereonine, serine and lysine residues. Formation of cross-linked compounds e.g. lysinoalanine also takes place ( Blackburn, 1968). Provansal *et al.* (1975) studied the effect of alkali treatment on sunflower protein and casein by heating them at pH 9.6, 9.8, 11.5, 12.7, and 12.9 at 55°C and 60°C for 1, 5, 15, and 16 consecutive hours. The content of aspartic and glutamic acids, alanine, leucine, methionine, methionine sulfoxide, phenylalanine, proline and tyrosine was not found to be significantly modified by the various treatments.

Progressive and marked losses in arginine, cystine, thereonine, serine, lysine and isoleucine occurred. Arginine was the amino acid most affected by this treatment with 100% loss after the most severe treatment. Losses in serine and therionine amounted to 55% and 82% respectively. A similar result was obtained by Blackburn (1968).

Alkali treatment of food proteins at a pH level of 12.2 and a temperature of 40°C for 4 hours decreased the content of cystine and to a lesser extent lysine. More drastic treatment at a temperature between 40-80°C also destroys serine and arginine (De Groot and Slump, 1969). The reduced cystine and lysine content was associated with the production of lysinoalanine (LAL) (De Groot and Slump, 1969; Provansal *et al.*, 1975). Destruction of cystine and lysine in alkali treated fish has also been reported by Carpenter *et al.* (1952). It is important to mention that when proteins are heated at natural pH levels, chemical changes also occur





(Bjarnason and Carpenter, 1970).

Some amino acids, namely isoleucine, leucine, tyrosine, phenylalanine, valine, alanine, and aspartic acid show increased content with drastic alkaline treatment (pH 12.2 and 40°C for 4 hours) (De Groot and Slump, 1969).

## 2.6 LYSINOALANINE (LAL)

### 2.6.1 Production of Lysinoalanine and Unusual Amino Acids

The alkali and heat treatment may induce protein modifications leading to the formation of new and unusual amino acids such as lysinoalanine (Patchornik and Sokolovsky, 1964; Bohak, 1964), lanthionine (Horn *et al.* 1941; Provansal *et al.*, 1975), ornithinoalanine (Ziegler *et al.*, 1967; Provansal *et al.*, 1975), and alloisoleucine (Provansal *et al.*, 1975). The formation of lysinoalanine (LAL) has been demonstrated in acid hydrolysates of various alkali treated proteins such as lysozyme, wool, silk and soyprotein isolate (Bohak, 1964; Patchornik and Sokolovsky, 1964; De Groot and Slump, 1969). LAL was probably formed by condensation of an  $\epsilon$ -amino group of lysine residue with a dehydroalanyl residue, leading to crosslinks within or between polypeptide chains (Bohak, 1964; Patchornik and Sokolovsky, 1964). The alkaline treatment of protein above a pH of 10.5 at 25°C or above pH 8 in boiling water causes the formation of LAL (Bohak, 1964). Effects of varying pH



levels, temperature and duration of treatment on the production of LAL was studied by De Groot and Slump (1969). LAL was present in samples treated at pH 12.2 and 40°C, or at room temperature for 4 hours. After 3 hours at pH 9 and 90°C, LAL was not detectable. The LAL content appeared to at first increase and then decrease as the severity of the alkaline treatment was increased. Pure LAL was partly destroyed when submitted to NaOH at 80°C for 16 hours (Provansal *et al.*, 1975).

Sternberg *et al.* (1975b) demonstrated the widespread occurrence of LAL in home-cooked and commercial foods and ingredients that may not have been exposed to alkali. Particularly significant was the finding of LAL in condensed milk, acid casein, cooked chicken thigh and sirloin steak pan scrappings, none of which were exposed at any time to an alkaline medium. Results presented by Sternberg *et al.* (1975b) seem to confirm that LAL does form in proteins under conditions that do not entail alkali treatment. Proteins occurring in food systems such as soya globulin, ovalbumin, lysozyme, casein and bovine serum were heated under nonalkaline conditions. All formed varying amounts of LAL in the range of pH, temperature and time commonly used in home cooking and commercial processing (Sternberg *et al.*, 1975a). Aymard *et al.* (1978) also found LAL to be omnipresent. They found that LAL is sometimes present in large amounts in most heated proteins. The lanthionine (LAT) content was lower than that of LAL.



Table 6 shows LAL and LAT contents of different proteins.

Aymard *et al.* (1978) also reported that there is no correlation between the lysine over cysteine + cystine ratio on the one hand, and the LAL over LAT ratio (calculated with the oilseed cakes) on the other hand. LAL has been found to develop spontaneously in a freshly laid egg after being stored for a few days (De Groot *et al.*, 1976a). From that we can surely assume that LAL has been a component of dietary protein since man first learned to cook (O'Donovan, 1976).

#### 2.6.2 Determination of LAL

The determination of LAL can be accomplished by ion exchange chromatography (De Groot and Slump, 1969; Robson *et al.*, 1967), high voltage electrophoresis (Asquith and Carthew, 1972), thin layer chromatography (Sternberg *et al.*, 1975a), and automatic amino acid analyses (Slump, 1977). Using the last technique, LAL is detectable at levels higher than 20 ppm of protein. The differences between duplicate analyses are 10-50% of the mean for LAL levels lower than 100 ppm and about 10% of the mean at levels higher than 100 ppm.

#### 2.6.3 Inhibition of LAL Formation

The addition of cysteine concurrently with the alkali treatment will favour the formation of LAT at the expense of LAL (Snow *et al.*, 1976). The addition of cysteine or other mercaptoamino acids probably prevents LAL formation by both







Table 6. Lysinoalanine and Lanthionine in Vegetable Protein Preparations.

Defatted oilseed cakes	LAL ( $\mu\text{g/g}$ protein)	LAT ( $\mu\text{g/g}$ protein)
Peanut	900	200
Soya	1500	300
Sunflower	900	300
Rapeseed	1500	100
Linseed	1400	150

From Aymard *et al.*, 1978.



trapping dehydroalanine residues to form LAT side chains and by reducing disulfide bonds to cysteine side chains, thus minimizing dehydroalanine formation (Finley *et al.*, 1978). Other organic and inorganic compounds such as mercaptoacetic acid, hypophosphite, and bisulfide, can be used as reducing agents. These reducing agents are effective in protecting the protein from LAL formation (Finley and Kohler., 1979). Oxygen is required for the formation of high levels of LAL. Careful control of the amount of air allowed in the system combined with reducing agents will effectively reduce the LAL formation to extremely low levels (Finley and Kohler., 1979). Although the use of reducing agents results in less LAL, care must be taken to prevent formation of other toxic by-products. This should be done through extensive animal testing of these products (Finley and Kohler., 1979).

#### 2.6.4 Toxicity of LAL

De Groot and Slump (1969) noted that under extreme alkaline treatment, net protein utilization and digestibility of the treated protein was reduced. Renner and Jelen (1980) showed similar relationship with alkali solubilized heated whey protein. Woodard and Short (1973) found a unique histological abnormality termed cytomegaly in the kidneys of rats fed either a partially hydrolyzed alkali-treated industrial protein or soya protein that had been subjected to severe and prolonged alkali treatment at an elevated temperature. They suggested that the use of



alkali-processing techniques employed in the food industry may have important toxicological implications. The enlargement of kidney cells of rats was reported also by several workers (Woodard and Alvarez, 1967; Woodard, 1969). De Groot *et al.* (1976a, b) have shown that rats are extremely sensitive to the nephrocytomegalic effects of LAL supplied in their diet either in the form of free synthetic amino acid or forcibly rendered from an alkali-treated protein by complete acid hydrolysis. The dietary addition of 100 ppm free synthetic LAL was consistently nephrocytotoxic to rats, while a dietary level of 30 ppm was without effect. On the other hand, the feeding an alkali-treated protein providing 2400 ppm protein-bound LAL was devoid of renal cytomegalic effect. De Groot and Slump (1969) did not observe any clinical or histological abnormalities other than an increased degree of nephrocalcinosis in the female rats, a condition which is already alleviated by adjustment of the level of phosphorus and calcium in the diet. The LAL produced nephrocytomegaly was found to be reversible if LAL is removed from the diet (Struthers *et al.*, 1977, 1978). However, there was no evidence to indicate whether the individual cytomegalic cells actually revert to normal, or die off, leaving the kidneys normal in appearance. The latter is more likely (Struthers *et al.*, 1978). De Groot *et al.* (1976a) were unable to induce the lesions in rats fed protein at levels providing 100-1200 ppm LAL. Swiss mice, Golden Syrian hamsters, New Zealand white rabbits, and





Japanese quail were fed diets supplemented with 1000 ppm synthetic LAL without any effect on growth rate, food intake or food efficiency, while histological examination of the kidneys after the feeding of these diets for 4 and 8 weeks failed to reveal renal cytomegaly or other evidence of visceral toxicity (De Groot *et al.*, 1976a). Male rhesus monkeys were fed diets containing either 1000 ppm free synthetic LAL or 10,000 ppm protein-bound LAL, provided by alkali-treated casein, for a period of 8 weeks without evidence of an adverse effect on growth. Neither renal cytomegaly nor other evidence of visceral pathology related to treatment was observed on microscopic examination (De Groot *et al.*, 1976b). According to O'Donovan (1976) there is strong evidence to suggest that LAL sensitivity is specific to the rat. This should give investigators a reason to pause before extending new and unusual toxicological observations from a single species to man, or before condemning any existing or new food processes that may use alkali treatment with proteinaceous food materials.



### 3. STATEMENT OF OBJECTIVES

This work is concerned with extraction of oil and protein from rapeseed. As indicated in the literature survey, the industrial prepress-solvent extraction of rapeseed is the method most widely used in Canada (Youngs *et al.*, 1978). According to the Fourteenth Report of the joint FAO/WHO expert committee on food additives (1971), the use of solvents in food technology raises four toxicological issues: (a) treatment with solvents may effect the nutritive value of foodstuffs; (b) residues of solvents may have toxic effects; (c) impurities in solvents may remain in the extracted food and have toxic effects; and (d) a solvent may react with constituents of a foodstuff to form toxic products. Along with the possible toxic effects the prepress solvent extraction has other drawbacks. Among these are the high prices of solvents and their volatility which could cause health and environmental hazards, such as fire and pollution. In addition, the worms of the press used in pre-pressing the seeds have to be changed regularly. The cost of replacement is very high (Unger, 1979).

Solvent-extracted crude canola oil is free of solvent, but contains impurities such as lecithin gums. Degumming equipment uses steam or hot water and a centrifuge to remove the gums yielding crude degummed canola oil. Oil extracted using the aqueous technique does not need the degumming process, and it is of a superior quality compared to the solvent-extracted oil (Embong, 1977).





The present conventional method does not have a provision for the removal of glucosinolates or their hydrolysis products. This lowers the quality of the produced meal and limits its use (Jones, 1979; Anjou *et al.*, 1978; Embong, 1977). These disadvantages underscore the need to develop a new processing technique. This technique should be economical. It should limit or eliminate the use of organic solvents, produce a high yield of high quality pure oil and provide a built-in system to remove or reduce glucosinolates. Embong and Jelen (1977) suggested a new method for the aqueous extraction of rapeseed oil employing a process for washing the oil out of crushed seed, at neutral pH. Their process required a prolonged heat treatment and several steps of dry and wet grinding. Although the produced meal had a considerably lower glucosinolates content it had a dark undesirable color. Also, the method did not suggest a way to utilize the valuable rapeseed protein for human consumption. The method of Embong and Jelen (1977) was explored on a small scale; thus their oil yield was high (above 90%). However, when the method was used with bigger batches the same high yield was not obtainable.

Sugarman (1956) patented a method for alkaline extraction of oil and protein from oleaginous materials. The method deals with unheated undenatured proteins and was applied successfully with soybean, peanut, tung nut and cotton seeds.



However, when it comes to rapeseed, heating is a necessary step in destroying the enzyme myrosinase to prevent contamination of the oil with sulfur. Heating of oil seeds to high temperatures during processing also results in partial denaturation of the protein, thereby decreasing the yield and quality of the protein which may be extracted (Sugarman, 1956).

The purpose of this research was to investigate the technical feasibility of developing an aqueous alkaline process to simultaneously extract oil, protein and meal from rapeseed. In the course of this work the following objectives were developed.

1. The modification of the aqueous extraction method (Embong and Jelen, 1977) in order to produce acceptable oil yield in mostly free and pure state, using the mildest possible treatment in the shortest time, meanwhile keeping the protein losses in the liquid fraction at a minimum.

2. The investigation of the processing parameters affecting the alkaline extraction of preheated rapeseed for oil and protein, and the evaluation of the effects of time, temperature and pH of the alkaline treatment of rapeseed on the quality of rapeseed protein and oil, along with the effect on the glucosinolates.

3. The optimum data obtained from the aforementioned research were used in formulating a technically acceptable two-stage process. The aim of the process was to maximize the removal of the oil and glucosinolates in the first stage



with losses of protein kept at a minimum. In the second stage, alkaline extraction was employed to extract as much of the residual oil and protein as possible. No attempt was made to study the breaking of the emulsion. This complicated technological problem was deemed to be beyond the scope of this work. However, Sugarman (1956), working with peanut, soybean, cotton seed and tung seed, and Hagenmaier (1974) with sunflower, have shown that the process of breaking an emulsion to produce pure oil and protein is technically achievable.





## 4. MATERIALS AND METHODS

### 4.1 RAW MATERIALS

Commercial rapeseed (canola) used in this investigation was claimed to be the triple low variety known as Candle, which is low in erucic acid, glucosinolates and fibre. Variety Candle is of the *Brassica campestris* species known also as Polish type rapeseed. Two batches of seeds were used. The first was supplied by United Oilseeds of Lloydminster, Alberta. It was used in the first part of this investigation which is related to the aqueous extraction process. The second batch was supplied by Alberta Food Products, Fort Saskatchewan, Alberta. This second batch was used in the rest of the investigation. Fig. 2 shows that the actual proportion of the yellow seeded Candle in the experimental material was fairly low.

The chemical composition of the two batches was similar. The first batch contained 42% oil, 7.5% moisture, 21.5% protein, 4.4% total ash, 2.9 mg/g total glucosinolates, and 13% crude fibre content. The second batch contained 42.3% oil, 6% moisture, 23% protein, 4.45% total ash, 2.9 mg/g total glucosinolates, and 13.5% crude fibre content. All of the above values were determined in full-fat seed, with the exception of the crude fibre and glucosinolates content which were determined and expressed in defatted dried meal.





Fig. 2. Canola sample supplied by Alberta Food Products (low percentage of yellow seeded candle).





Other materials were also used in this investigation at various stages for various purposes. Their origin and identification were as follows.

*Enzyme deactivated rapeseed flakes*: rapeseed which was industrially flaked and heat treated in order to destroy the enzymes. It was supplied by Alberta Food Products.

*Rapeseed cake*: residue of rapeseed after removing 70-80% of the oil using expeller. It was supplied by United Oilseeds of Lloydminster, Alberta.

*Industrial rapeseed meal*: This term denotes, for our purposes, the solid residue left after the prepress-solvent extraction of oil. This meal was prepared from the same batch of rapeseed supplied to us by Alberta Food Products of Fort Saskatchewan, Alberta.

*Soxhlet extracted meal*: the solid residues left after oil extraction by soxhlet method in our laboratory.

*Industrial crude oil*: crude rapeseed oil supplied by United Oilseeds. It was obtained using the pre-press solvent removal extraction method.

#### 4.2 PROCESSING

Fig. 3 represents a simplified flow chart for the two step aqueous alkaline extraction of rapeseed as adopted from our experiments. All experiments were performed at least in duplicate. Amount of ground rapeseed used with every batch was 200 gm.



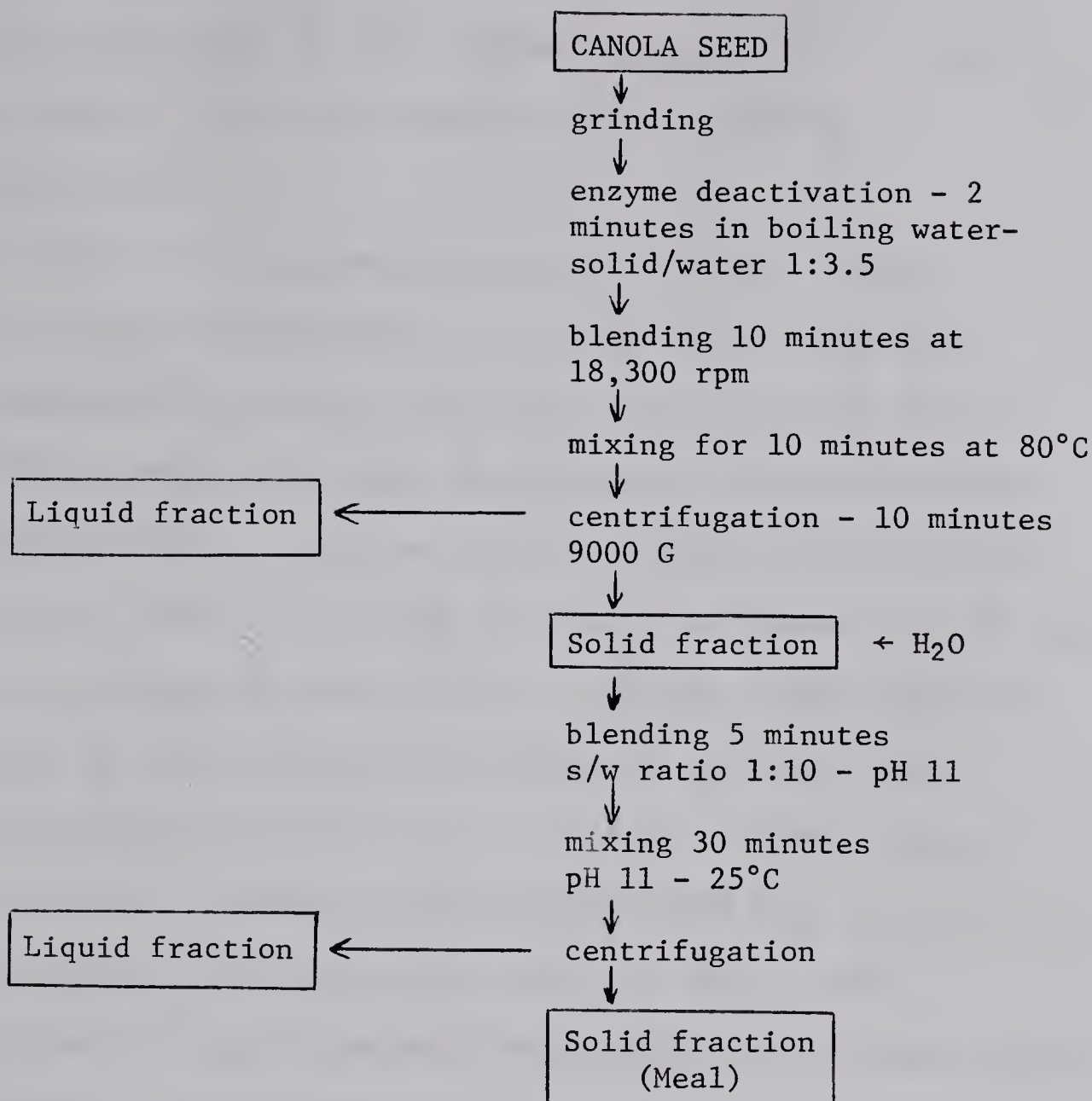


Fig. 3. Flow chart of the final two stage extraction procedure.



#### 4.2.1 Grinding

The grinder used in this investigation was a rotating disc type Quaker City mill (Model 4-5, Straub Co., Philadelphia, Pa.).

Grinding is a very important and critical step. Grinding of rapeseed presents a universally recognized problem because of the seed size and the high oil content (Anjou, 1972). The cells must be ruptured to release their constituents and to increase the efficiency of extraction (Cater *et al.*, 1974). The only grinder available for use was not entirely suitable. The grinding assembly consisted of two vertical discs. The distance between the two discs determined grinding efficiency. Very loose discs did not rupture the cells, whereas tight discs produced very sticky overheated paste. The operation was very slow. The overheated seeds later produced very dark colored meal. The grinder did not have a provision to control the particle size. However, we ran some batches when the grinder discs were somewhat loose and also when they were very tight. The oil yields were 46 and 55.5%, respectively. A healthy increase of about 10% indicates the effect of efficient grinding on oil yield. The above increase in yield coincided with the production of meal with a darker color.

Embong's meal had a very dark color (Bourgois, 1979), resulting from the severe grinding treatment which might have increased his oil yield but at the expense of protein color and quality (Embong, 1977).





For our work the tightness of the grinder discs was regulated in order to prevent discoloration of the seeds which were passed through the grinder twice. Wet grinding was not applied because the rapeseed slurry was leaking from the back of the grinder making it very difficult to control and calculate the yield. A good grinder with a built-in cooling system and with a provision for regulating and measuring the particle size would be invaluable. It would then be possible to determine the optimum grinding conditions. Excessive grinding could produce smaller oil globules which make breaking the emulsion more difficult. Insufficient grinding results in large losses of oil in the residue (Cater *et al.*, 1974).

#### 4.2.2 Enzyme Deactivation

Enzyme deactivation was performed by dispersing the ground rapeseed in a stainless steel beaker containing boiling distilled water. The temperature was maintained at the boiling point for two minutes before cooling by immersing the beaker in a tank of cold, running water. The addition of the ground seed to the boiling water was conducted with vigorous agitation to avoid any enzyme activity as a result of the possible existence of some cold, wet spots.

No dry enzyme deactivation was performed in our laboratory. The ready-flaked, enzyme deactivated seed was used to study the effect of dry heat on the extraction of



oil and protein. The effectiveness of the wet enzyme deactivation step adopted by our laboratory was studied by analyzing the treated seed for the hydrolysis products of glucosinolates. The analyses were performed at the Department of Plant Science, University of Alberta, using the original thiourea method of McGregor, which was adopted from Wetter and Youngs (1976).

#### 4.2.3 Blending

The enzyme deactivated rapeseed slurry was blended for ten minutes in a three-speed, 3.8 L commercial waring blender (Waring Products Co, New Hartford, Connecticut 06057). Unless otherwise indicated, the grinding speed was 18,300 rpm.

In some experiments, a "polytrone" homogenizer (Brinkman Instruments, Rexdale, Ontario) was tried as an alternative step, but it was found unsuitable because it produced a very stable, hard to break emulsion. In experiments when extraction was carried out at a certain pH level, the pH of the slurry was readjusted prior to blending. The pH usually changed during blending and was readjusted before and during the extraction step.

#### 4.2.4 Mixing(Extraction)

In this step, extraction was conducted by mixing the blended sample using a propeller stirrer. The stainless steel beaker containing the blended rapeseed slurry was





placed in a water bath. The water bath temperature was controlled by a heater circulator Thermomix 1441 (B. Braun Melsungen).

Fisher Stedi-speed Stirrer, Model 12, with speed setting control (Fisher Scientific) was used. The Fisher Stedi-speed Stirrer monitors the viscosity during stirring and automatically adjusts the motor power as the load changes. Thus, the selected speed was maintained at a constant rate. The stirrer speed was adjusted at 2000 rpm. The beaker was covered with a semi-tight plastic cover with a hole in the middle for the stirrer rod. This cover helped to keep water losses through the mixing to a minimum (Fig. 4). Before and during mixing the pH of the mixture was checked and adjusted every five minutes. Although the stirrer has a provision for speed as high as 3200 rpm, a speed of more than 2000 rpm was not used because of the possibility of air incorporation and its undesirable effect especially under alkaline conditions (Finley and Kohler., 1979). For extraction at the natural pH, the solid to water ratio of 1:3.5 was used. For the extraction under alkaline conditions the S/W ratio of 1:10 was adopted after some experimentation(5.2.1).

#### 4.2.5 Adjustments of pH

The pH of the rapeseed slurries was maintained at the desirable values. It was adjusted before and after blending, also every five minutes during mixing, by 20%





Fig. 4. Extraction apparatus



sodium hydroxide or hydrochloric acid. A pH meter, Fisher, Model 230, pH/ion meter (Fisher Scientific) was used.

#### 4.2.6 Centrifugation and Drying

After mixing, the rapeseed slurry was centrifuged at 9000 g for ten minutes in 250 ml bottles, using Beckman centrifuge, Model J21B, Rotor JA14. After centrifugation, the supernatant including the oil emulsion was separated from the solids, thus producing liquid and solid fractions. Both fractions were freeze dried to approximately 4% moisture contents using Virtis Freeze-drier (Virtis Co. Gardiner, New York) before analyses were performed.

#### 4.2.7 Yield Calculation

The freeze dried liquid and solid fractions were analyzed for oil and protein content. A mass balance was calculated using the following equation:

$$\% \text{ of oil extracted} = \frac{x}{x + y} \times 100$$

whereas

x = gram of oil in liquid fraction

y = gram of oil in solid fraction

The same equation was used with other components. In any given batch, if the amount of oil or protein unaccounted for due to laboratory inefficiency exceeded 5%, the batch was disregarded and additional batch was run. The percentage





of oil left in the dried meal (solid fraction) was also used as an indication of extraction efficiency in a few preliminary experiments.

The free oil content was determined by carefully pipetting the oil out of the centrifuge bottles and collecting it in a preweighed aluminum dish. The dish and content was dried under vacuum for 30 min at 125°C before it was cooled and weighed. The weight of the free oil was related to the weight of the total extracted oil.

#### 4.2.8 Determination of Viscosity

The viscosity of rapeseed slurry at different pH levels was determined at 25°C and 70°C using a Brookfield Synchro-Lectric Viscometer, Model RVT (Brookfield Engineering Laboratories, Stoughton, Mass., U.S.A.). The determination took place after stirring was completed. Spindle #5 was used at a speed setting of 1/20. The readings taken from the viscometer were used to make direct comparisons between the different slurries, without determining exact viscosity values.

#### 4.2.9 Effect of Alkaline Conditions

The samples were prepared by blending the enzyme inactivated ground rapeseeds for 10 minutes at the desired pH, water to solid of 1:10 and blender speed of 18,300 rpm. Mixing took place at 2000 rpm for one hour at the investigated pH values and temperatures which were



maintained throughout mixing.

After mixing was finished, each batch was divided into two equal parts. The pH of one part was readjusted to the original pH of rapeseed (5.8) before it was freeze-dried. Oil was removed by soxhlet and was tested for the free fatty acid content. On the defatted meal, tests for glucosinolates content, color determination, lysinoalanine formation, amino acid profile and gel electrophoresis pattern were performed. The second part was centrifuged. After separation, the pH of the solid and liquid fractions was readjusted to the original pH of repressed (5.8). Liquid and solid fractions were tested for glucosinolates. Color was determined in the liquid fraction alone.

### 4.3 ANALYTICAL PROCEDURES

#### 4.3.1 Analyses of Crude Oil

Most of the investigation concerning the effects of aqueous extraction on oil quality was covered by Embong and Jelen (1977). Their work showed the superiority of the aqueous extracted oil. However, because of the high pH used in most of our experiments, analysis of the possible fat hydrolysis to free fatty acids was necessary in this work. Determination of free fatty acids content was performed as described in A.O.A.C. (1975).



#### 4.3.2 Analyses of Meal and Aqueous Fraction

The liquid fraction and the meal were both freeze-dried. Oil was removed by soxhlet extraction. With the exception of oil determination all tests were performed on moisture-free and fat-free materials. All the analyses were performed according to A.O.A.C. (1975).

- a) *Oil*: The oil contents of the freeze-dried extracts were determined by extraction in a soxhlet apparatus using petroleum ether (b.p. 30-60°C) for eight hours or overnight.
- b) *Nitrogen*: The nitrogen content of the defatted materials was determined using the microkjeldahl method. The factor 6.25 was used to convert the nitrogen to crude protein.
- c) *Total Ash*: Total ash was determined using a furnace at 600°C.
- d) *Crude Fibre*: The crude fibre content determination was carried out at the Animal Science department, University of Alberta. The determination was performed according to the A.O.A.C. (1975), modified by the use of a cloth filter instead of asbestos.

#### 4.3.3 Amino Acid Determination Including Lysinoalanine

The chromatographic analysis of amino acids including lysinoalanine (LAL) was carried out in the Department of Biochemistry, University of Alberta, on a Dionex D500 Amino Acid Analyser, formally known as Durrum (Durrum Instrument Corporation, Palo Alto, Ca.).







A column 50 cm long and 1.75 mm in diameter was packed with 8±2 micron Durrum type 6A resin 8% cross-linked. The column was operated under back pressure between 2000 and 2500 psi with a buffer flow rate of 10 ml/h.

Standard solution of lysionoalanine was run independently to determine the factor to be used for calculating the concentration. Calibration standard + LAL were also run to determine location of LAL and to help in achieving good separation of LAL from histidine (Friedman, 1979).

Amino acid hydrolysate was prepared as follows: About 5 mg of the sample was hydrolyzed with 5 ml of 6N HCl at 110°C for 22 h in a vacuumed, sealed glass tube. The hydrolysate was evaporated to dryness in a vacuum dessicator in the presence of NaOH pellets. The dried hydrolysate was dissolved in a sodium citrate buffer at pH 2.2.

#### 4.3.4 Determination of Total Glucosinolates

The total glucosinolates content of freeze-dried liquid and solid fractions was determined at the Plant Science Department using the routine methodology of McGregor, which is modified from Wetter and Youngs (1976).

In the McGregor's method the defatted, heated rapeseed meal is treated with the enzyme myrosinase at pH 7, which favors the formation of isothiocyanates from glucosinolates. The isothiocyanates are then extracted by methylene chloride and are converted to their substituted thiourea derivatives



by reaction with ammonia in ethanol. Those isothiocyanates which contain a hydroxyl group in their side chain cyclize to form oxazolidinethiones. Quantitative measurement of thiourea derivatives and/or oxazolidinethiones were conducted using ultraviolet spectrophotometry at 245 nm.

#### 4.4 SODIUM DODECYL SULFATE (SDS) POLYACRYLAMIDE GEL ELECTROPHORESIS

The SDS-polyacrylamide gel electrophoresis preparation was according to the procedure of Porzio and Pearson (1977). The system utilizes a running gel consisting of 10% acrylamide with 0.1% bisacrylamide (100:1) incorporating 400 mM tris/glycine (pH 8.8), 0.1 mM methylenediaminetetraacetate, 5% glycerol and 0.1% sodium dodecyl sulfate. Samples containing 100 mg of protein in 1.25 mM EDTA and 30% glycerol were applied in a volume of 25 ml. Electrophoresis was performed at 0.75 mA per gel using a Buchler disc-gel apparatus (Buchler Industries, Fort Lee, NJ). Running time was nine hours. The gels were fixed by soaking in a solution of 25% (v/v) isopropanol/10% (v/v) acetic acid for several hours. The samples were stained overnight in 0.01% coomassie Brilliant Blue R250 according to Weber and Osborn (1969). The molecular weight of rapeseed protein was determined by comparing the relative mobility of these proteins to standards of known molecular weight and relative mobility (Nagainis, 1981).



#### 4.5 DETERMINATION OF COLOR

Along with color photographs taken by a Pentax ES 11 camera, a Hunterlab Colorimeter, Model D25L-2 (Hunter Associates, Inc., Fairfax, Virginia, U.S.A.) was used to quantify the effects of different alkaline and heat treatments on the color of the rapeseed meal and protein concentrate.

The Hunter L, a, b scale values were used to compare the effects of various treatments. Of the three values obtained by this measurement the L value (lightness) was deemed the most important indicator. At least two different batches of the experimental materials were used for all the measurements. Each reading within a batch was repeated at least three times. The differences between replicate readings within a batch were negligible.





## 5. RESULTS AND DISCUSSION

This study was divided into four major parts. The first part was concerned with the unit operations constituting the first stage of the extraction process. Investigations were conducted in order to wash out as much oil and glucosinolates as possible. Simplicity of the process as well as minimizing protein losses were also taken into consideration.

The second part included the evaluation of the various factors affecting the alkaline extraction of oil and protein from heated rapeseed at pH 8, 9, 10 and 11.

The third part dealt with some physico-chemical effects of various heat and alkaline treatments on the oil, protein and glucosinolates of the treated rapeseed.

In the fourth part, information obtained from the above investigation was used to formulate and test a two-stage aqueous alkaline process.

It is rather important to mention that although the process of Embong and Jelen (1977) was used as a base for this investigation, our approach was quite different. Embong and Jelen (1977) concentrated on the absolute maximization of oil yield in one step regardless of the economic considerations, and of the possible effects of heating for one hour at 70°C on the non-enzymatic reactions which is known to have undesirable effect on the protein (Rutkowski, 1970).



In our preliminary work, the unit operations used by Embong and Jelen (1977) were studied in an attempt to improve the efficiency bearing in mind time and economical factors. It was found necessary to develop our approach based on further studies of the essential unit operations.

## 5.1 SELECTION OF THE OPTIMUM PROCESSING CONDITIONS

### 5.1.1 Blending

Blending was not used in aqueous extraction of coconut, peanut and sunflower seed oil (Hagenmaier *et al.*, 1972; Rhee *et al.*, 1972; Hagenmaier, 1974). However, it may be necessary for rapeseed because of the high fibre content. Embong and Jelen (1977) studied the effect of blending on the aqueous extraction of rapeseed oil. The percentage of extracted oil rose from 65% to about 82% after 5 minutes of blending, and about 88% after 15 minutes. Further blending for 15-35 minutes increased the amount of oil extracted by only 2%.

We duplicated Embong and Jelen's (1977) work with the elimination of the wet grinding step (thus, the sequence was ,grinding-boilling-blending at 7.3, mixing at pH 6.6, 70°C for one hour before centrifugation). Blending was done at the speed of 18,300 rpm for 0, 5, 10, and 15 minutes. The results shown in Table 7 indicate the importance of blending since blending for 5 minutes significantly ( $p \leq 0.05$ )





Table 7. Effect of blending on oil yield.\*

Blending time(min)	Oil yield %	Free oil %
0	37a	88d
5	49b	79e
10	54c	72f
15	56c	69f

\*Means of three determination. Figures in the same column not followed by the same superscript are significantly different ( $P \leq 0.05$ )



increased the yield by 12%. Another five minutes of blending increased the yield by further 5% ( $P \leq 0.05$ ), while an additional five minutes blending did not cause a statistically significant increase in oil yeild. These results showed the same trend as was found by Embong and Jelen (1977) with the inclusion of wet grinding.

The above results indicate the inefficiency of the grinder used. Blending increased the yield; this might have been due to further break-down of the cellular structure. Increasing the blending time was coupled with the reduction in the percentage of free oil, from 88% with no blending to 68% after 15 minutes of blending. The reduction in the percent of free oil resulted from the formation of stable emulsion which needs to be broken later. Blending generated heat. The temperature of samples blended for 10 minutes varied from 78-85°C.

Our yield was not as high as the yield reported by Embong (1977). However, the results followed the same pattern. It is important to mention that blending plays a more important role during the alkaline extraction than during the aqueous extraction at neutral pH.

The use of Polytrone caused the formation of a very stable emulsion which could not be separated from the meal after centrifugation. This resulted in higher oil loss in the meal. The oil yield was reduced to about 20% with very little free oil. Blending was used mainly to complement the poor efficiency of the grinder. An efficient and suitable



grinder such as the model now available in the food science department, University of Alberta (Model JT, Homoloid machines, the Fitzpatrick Co, Elmhurst, Illinois 60126) might solve this problem. With more efficient grinding, possible changes in the process could involve enzyme inactivation by dipping the intact seed in boiling water and then cooling down before wet grinding for two or three times in the above machine. This might reduce the blending time and could possibly eliminate the blending step entirely. However, this has to be studied in detail.

Under the circumstances of the experiment we selected blending for 10 minutes as our recommended treatment. It was used throughout the remainder of the work. This was justified by the fact that another five minutes of blending increased the oil yield by a mere 2% and reduced the percentage of free oil. Also, the temperature of the slurry after 10 minutes of blending was as high as 85°C. Another five minutes of blending will expose the protein to unnecessary high overheating.

#### 5.1.2 Effect of pH on Mixing

The extraction process was accomplished by agitating the blended rapeseed slurry to enhance the separation of the seed constituents. The efficiency of the mixing step can be expected to depend on the viscosity of the system.

The effects of pH on the viscosity of rapeseed slurry, and the corresponding yield of rapeseed oil and protein in





the aqueous fraction is shown in figures 5,6 and 7. In this experiment, the pH of the enzyme deactivated rapeseed slurry was maintained between pH 4 and 11 before and after blending for 10 minutes, and also during stirring for one hour at 70°C.

The solid to water ratio used in the extraction was 1:3.5. Increasing the pH caused a large increase in the slurry viscosity after blending and during stirring. Fig. 5 shows the effect of pH on the relative viscosities of the slurries before centrifugation when measured at 25 and 70°C. The curve shows a sharp increase in the slurry relative viscosity at pH higher than 9. At pH 10 and 11 the slurry was too thick. It was obvious that the ratio 1:3.5 was not suitable for alkaline pH. The increase in the relative viscosity was probably due to the effect of alkaline conditions in dissolving the protein and its effect on protein swelling. Swelling can be defined in terms of the spontaneous uptake of a solvent by a solid. Earlier studies of soybean protein isolates, sodium caseinate, whey protein concentrate, heated whey protein, and meat protein extracts (Hermansson, 1972, 1973; Jelen and Schmidt, 1976; Jelen *et al.*, 1979) showed that swelling is strongly dependent on pH and ionic strength. The fluid uptake by a protein was found to increase with the increase in pH and temperature. Heating the protein dispersion caused the formation of a gel. A good correlation was found between gel strength and swelling ability (Hermanson, 1973). The increase in viscosity of our



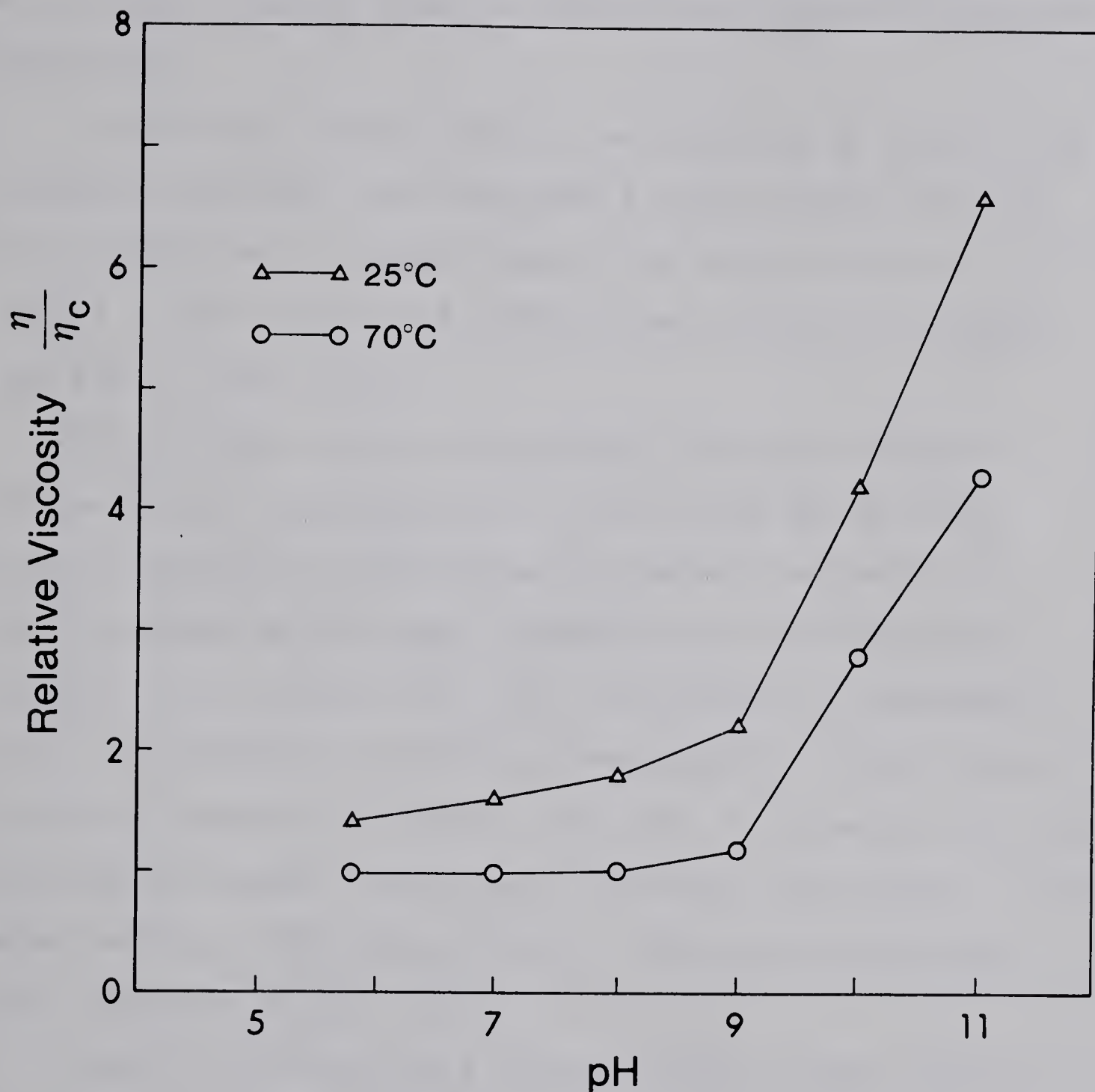


Fig. 5. Effect of pH on relative viscosity of rapeseed slurry during aqueous extraction. solid/water ratio = 1:3.5.  $\eta_c$  = reading at natural pH (5.8) and 70°C.





rapeseed protein slurries can be explained in the light of the above findings of Hermansson. In a later part of this investigation we found the solid-to-water ratio of 1:10 to be the most suitable when extraction took place at alkaline conditions.

At pH 5 the liquid fraction was clear with very little insoluble materials and contained a large volume of free oil. At pH lower or higher than 5 the liquid fraction started to get cloudy and turbid. The turbidity increased with the increase in pH.

The nitrogen solubility profile (Fig. 6) showed a minimum in the region of pH 5, which is in the minimum solubility region as determined by several workers for native protein at different temperatures (Gillberg and Tornell, 1976, Yang *et al.*, 1978, Korolczuk and Rutkowski, 1971). The nitrogen solubility increased on either side of the pH of minimum solubility. This was in agreement with the findings of several researchers (Gillberg and Tornell, 1976; Radwan and Lu, 1976; Yang *et al.*, 1978; Quinn and Jones, 1976; Thompson *et al.*, 1976).

The oil yield showed a minimum (48%) at pH 5 (Fig. 7). The yield increased when the pH of extraction was reduced to 4. In the alkaline direction, the yield reached a maximum at pH 9 before it dropped again. The decline in yield at pH above 9 was the result of the very high viscosity of the 1:3.5 slurry which hampered the separation during centrifugation thus leaving more oil in the solid fraction.



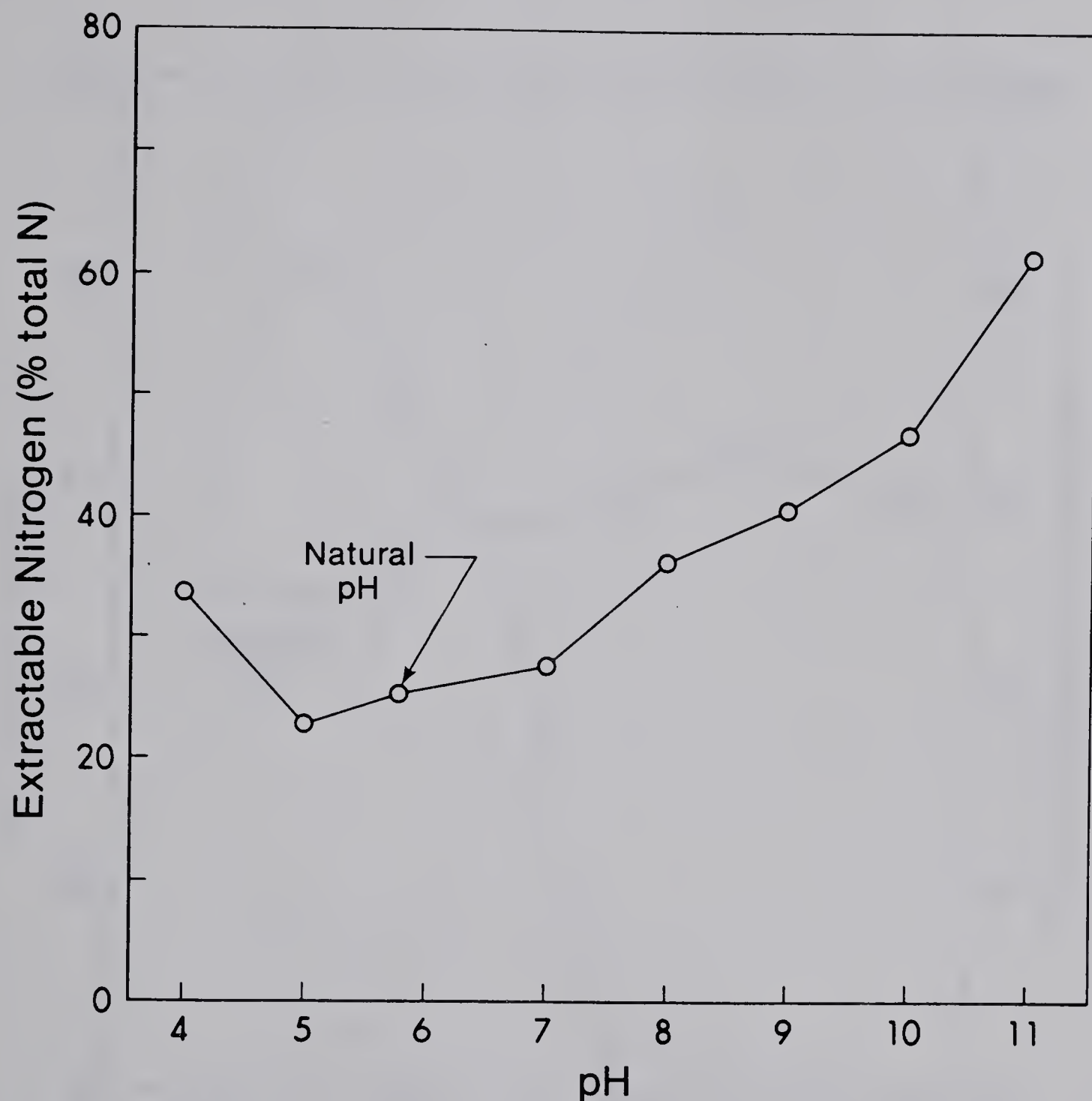


Fig. 6. Effect of pH on nitrogen extractability during the aqueous extraction. (solid/water ratio 1:3.5, enzyme inactivation 2 min in boiling water, blending time 10 min, mixing 1 h at 70°C)



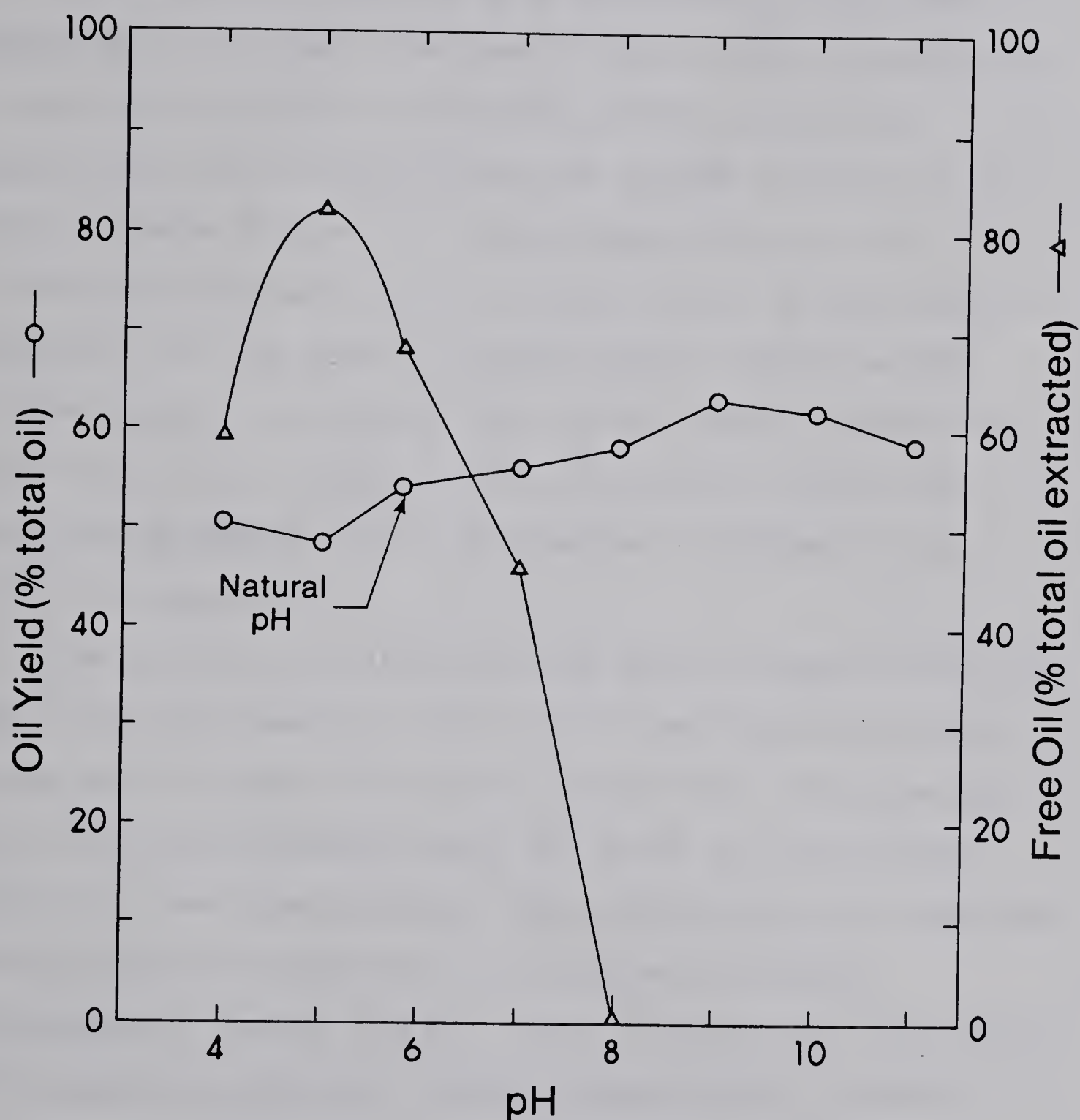


Fig. 7. Effect of pH on oil yield during the aqueous extraction of rapeseed. (solid/water ratio 1:3.5, enzyme inactivation 2 min in boiling water, blending time 10 min, mixing 1 h at 70°C)





In a later study at lower s/w ratio (1:7 and 1:10), the increase in protein yield was followed by an increase in oil yield even at pH 10 and 11.

The amount of free oil as a percentage of the total extracted oil reached a maximum of 82% in the region of pH 5, which is the region of minimum protein solubility. Changing the pH in either direction caused a reduction in the percentage of free oil. At pH 8 and above, no free oil was detected, and all the oil extracted was in the form of emulsion. The high free oil percentage at the pH of the minimum protein solubility might be the result of the low stability of the emulsion at the isoelectric point, as described by Embong (1977) for rapeseed and Rhee *et al.* (1973) for peanuts.

The color of the slurry at the pH of minimum solubility (pH 5) did not change. At pH 6 and 7 the slurries acquired a golden yellow color which again changed to a dark greenish color with the increase in pH. The color of the extract showed the same discoloration. The discoloration of rapeseed during alkaline extraction was reported by several investigators (Radwan and Lu, 1976; Rutkowski and Korolczuk, 1974; Sosulski and Bakal, 1969; Kodagoda *et al.*, 1973b).

Based on these results, extraction at the natural pH (5.8) was selected as the first extraction step. Among our reasons was the fact that no pH adjustment was needed, thus simplifying the process and eliminating the need for an acid or an alkali. Also the percent of free oil and total oil



yield were acceptable and the whole process was very simple.

### 5.1.3 Effect of Extraction Temperature and Time

To study the effect of temperature on extraction of oil and protein, the ground deactivated seed was blended for 10 minutes at the natural pH (5.8) before it was mixed at 2000 rpm for half an hour at temperatures of 25, 40, 55, 70 and 80°C. Fig. 8 shows the effect of temperature on the percentage of nitrogen extracted in liquid fraction. The percentage of nitrogen extracted at 25°C was 25.1% which increased with the increase in the extraction temperature to 26.3% at 40°C to reach a maximum of 29.6% at 55°C before it dropped to 22.6% at 70°C and reached a minimum of 15.8% at 80°C. The increase in nitrogen solubility with temperature was reported by several investigators for temperatures of up to 55°C. (Radwan and Lu, 1976). The decrease in the percentage of extracted nitrogen in the aqueous fraction with further increase in temperature may have been due to additional protein denaturation at all temperatures above 55°C, possibly combined with the observed emulsion breakdown.

As indicated in Fig. 9, the oil yields at the different temperatures were similar while the percentage of free oil increased substantially with the increase in temperature.

Extraction at 80°C was selected because it gave the highest percentage of free oil, the least percentage of nitrogen losses in liquid fraction and an acceptable total



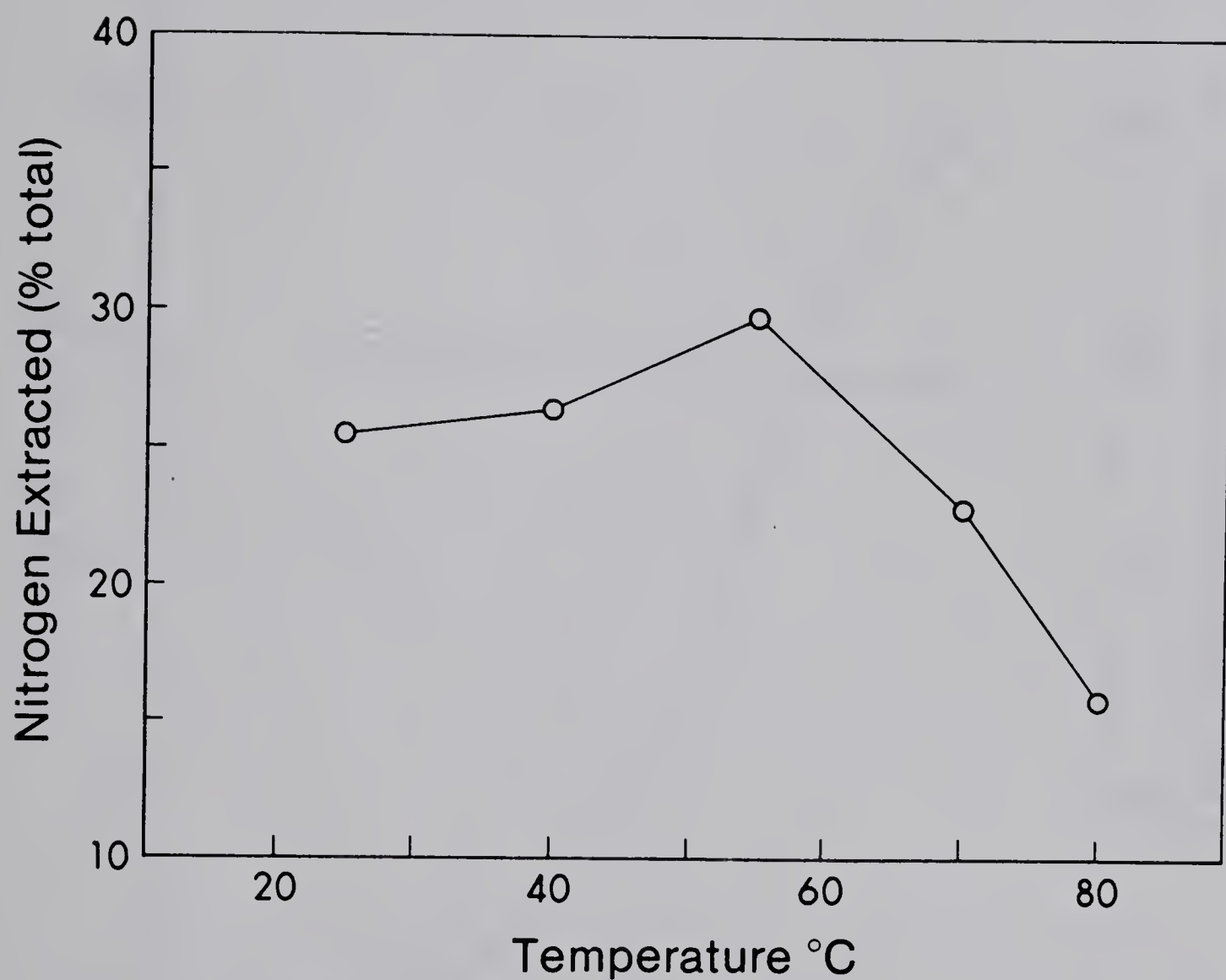


Fig. 8. Effect of extraction temperature on the percentage of nitrogen extracted in supernatant. (natural pH (5.8), s/w ratio 1:3.5, mixing time 30 min)





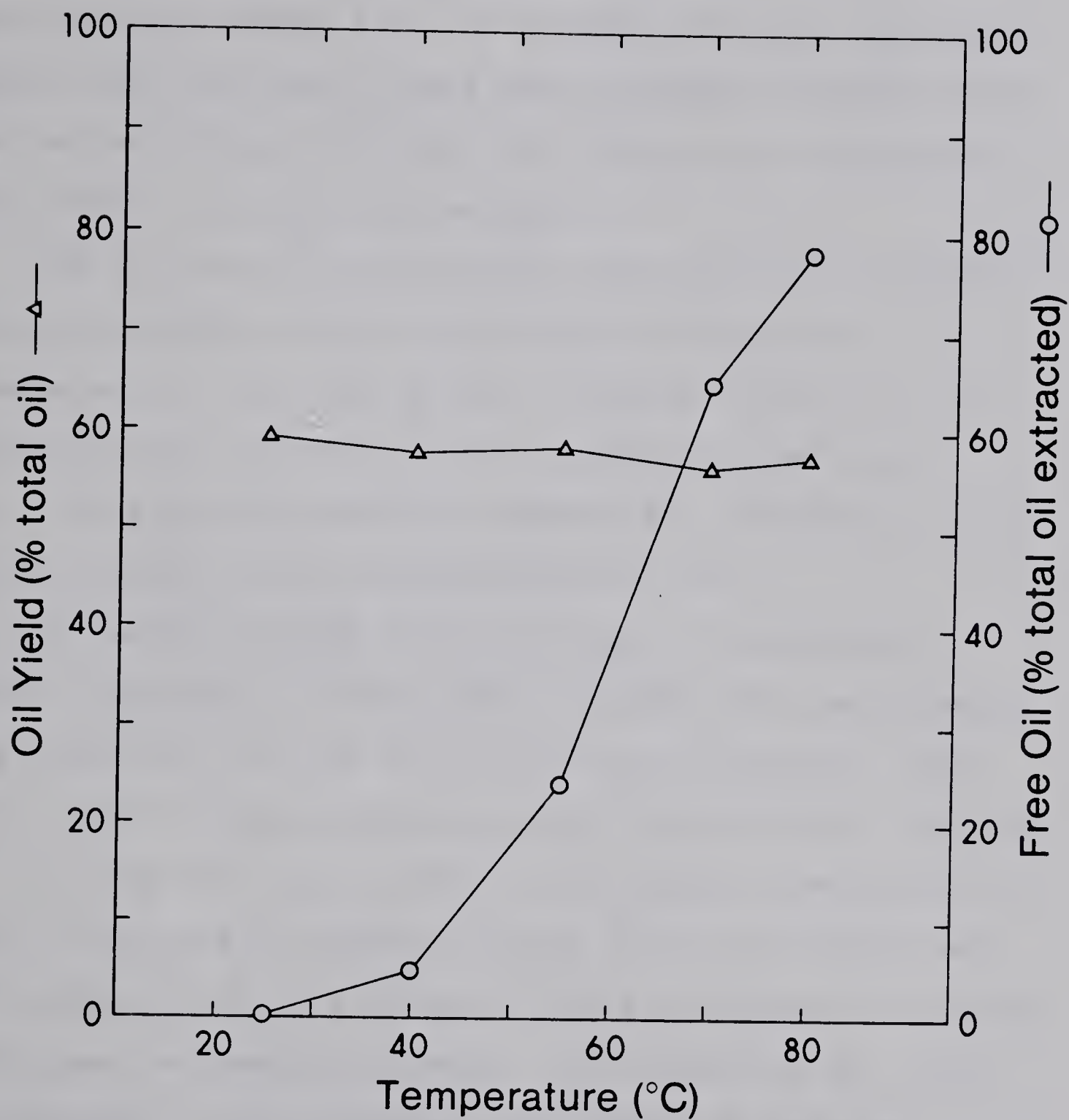


Fig. 9. Effect of extraction temperature on oil yield. (natural pH (5.8, s/w ratio 1:3.5, mixing time 30 min)



yield of oil.

Embong and Jelen (1977) conducted an extensive investigation on the effect of stirring time on oil yield. Their findings showed that the increase in yield took place only in the first hour. There was no further increase after four extra hours of stirring. The increase was significant only within the first half an hour.

Due to the fact that we were anticipating a two-stage extraction method, even half an hour of mixing was considered too long. The effect of shorter mixing time on extraction was explored. In this experiment the enzyme deactivated ground seeds were blended for 10 minutes before mixing at 80°C and at the natural pH of 5.8.

The results showed a statistically insignificant ( $P \leq 0.05$ ) decrease in extractable nitrogen with the increase in extraction time from 10 to 30 minutes (Table 8). There was a slight but statistically significant ( $P \leq 0.05$ ) increase in oil yield with the increase in extraction time: from 53.1% after 10 minutes of mixing to 55.3% after 20 minutes, and 57.2% after 30 minutes (Table 8). This was reported by other investigators (Embong and Jelen, 1977; Cater *et al.*, 1974). The increase in oil yield was followed by an increase in the percentage of free oil from 73% after 10 minutes of mixing to 77% after 20 minutes, and 78% after 30 minutes. This was also in agreement with the findings of Embong and Jelen (1977).



Table 8. Effect of mixing time at 80°C on oil and protein extraction\*.

Mixing time per min.	% of Nitrogen extracted** (lost) in liquid fraction	% of oil extracted in liquid fraction	% of free oil
10	16.3a	53.1b	73e
20	16.1a	55.3c	77f
30	15.8a	57.2d	78f

\*means of three readings.

Figures in the same column not followed by the same superscript are significantly different ( $P \leq 0.05$ )

\*\*in fat free moisture free samples





#### 5.1.4 Effect of Repeated Washing of Residue on Oil Yield

Repeated washing of residue was investigated for aqueous extraction of coconut (Hagenmaier *et al.* 1972) and sunflower (Hagenmaier, 1974). They reported a progressive decrease in the oil content of the residue with an increase in the number of times it was washed.

In order to investigate the effect of repeated washing on the aqueous extraction of rapeseed, 1200 gm batch of stirred rapeseed slurry was prepared and centrifuged using six centrifuge bottles. These were filled with an equal weight (230 gm) of the slurry. After centrifugation, one bottle was taken for analysis of the liquid fraction and the residue. From the five remaining bottles, the liquid fraction was removed and 75 ml of fresh distilled water was added. The contents of the bottles were mixed before they were centrifuged again for five minutes at 9,000 g. The process was repeated for a total of four washes.

The repeated washing of the residue with distilled water, even in this simple fashion was found to increase the oil yield. Fig. 10 shows a progressive decrease in oil content of the residues with the increase in the number of times the residue was washed. The percentage of oil in dried residue decreased from 30.7% without washing to 10.11% after four washings. This increased the oil yield from about 55% to about 88%. The decrease of the oil content in the residue was followed by an increase in the percentage of protein extracted in the water used in washing. As shown in Fig. 10



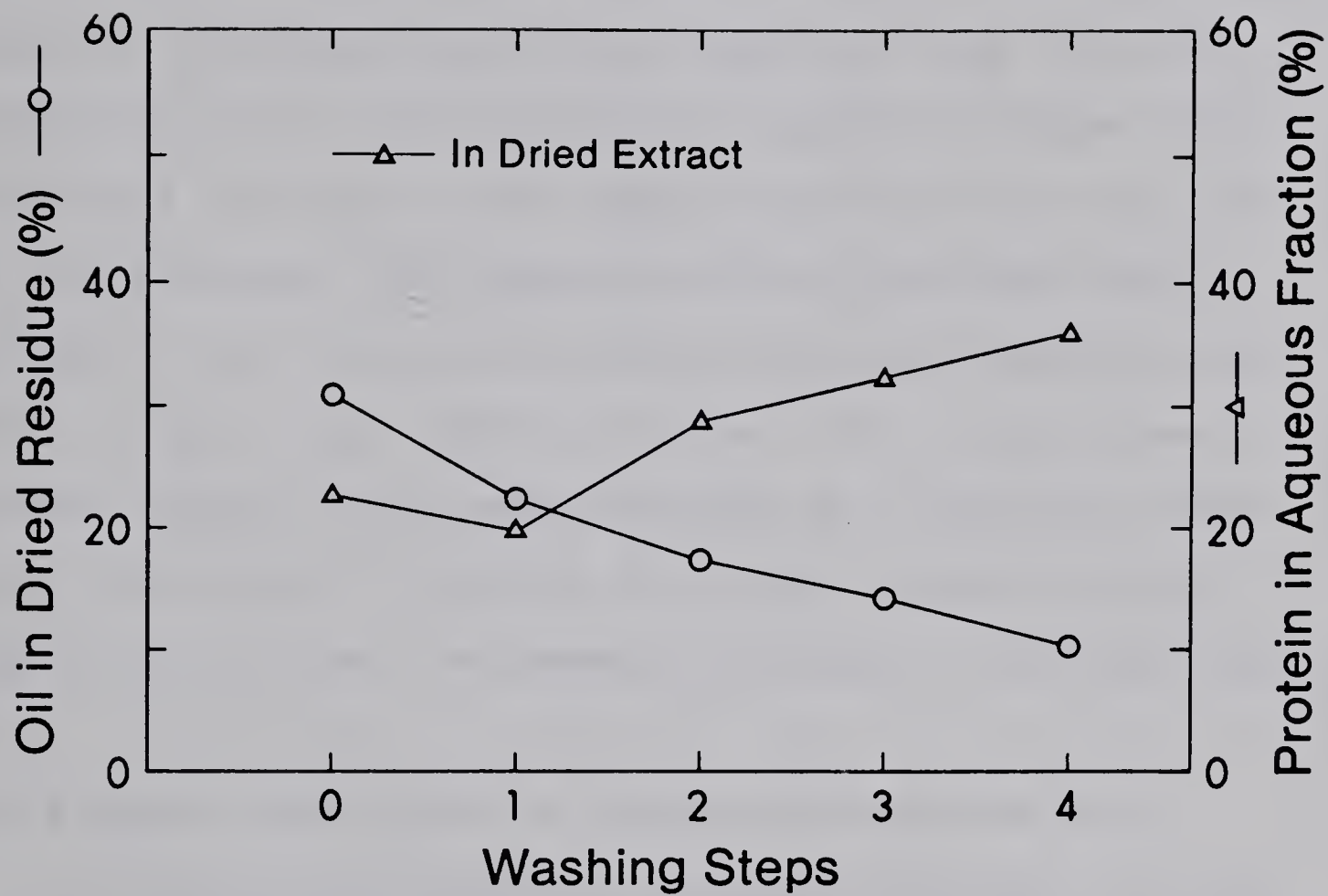


Fig. 10. Effect of multiple washings on oil and protein extraction.



the protein content of the dried liquid fraction increased from 19.9% after one wash to 35.4% after the fourth wash.

The natural pH of the residue increased gradually from 6.6, which was the pH of the original slurry, to about pH 7.4 after the fourth washing. The liquid fractions of all the washings seemed turbid and cloudy. They did not contain free oil, and seemed more like suspension than solution. Washing at pH 4.8 which is in the region of minimum protein solubility, produced clear liquid fraction with traces of oil and proteins. The repeated washing technique was not pursued further because its adaptation for industrial use would involve large volumes of water, which would present a problem. Also, the oil was extracted as an emulsion which would have had to be broken. This would involve further complication as well as losses of protein in the wash water.

#### 5.1.5 Aqueous Extraction of Industrial Rapeseed Cake

In several experiments, industrial rapeseed cake was used as a starting material. The rapeseed cake which was easy to grind contained about 18.8% of oil. After aqueous extraction using a 1:5 s/w ratio, the oil content of the residue was 17.2%. Several trials showed that it was hard to remove the oil from industrial rapeseed cake. It was concluded that there would be no advantage in developing the aqueous process in conjunction with the pre-press step of the current extraction process.





## 5.2 EXTRACTION UNDER ALKALINE CONDITIONS

In order to establish a technologically feasible process for the alkaline extraction of oil and protein from preheated rapeseed, several parameters had to be studied. The effects of the alkaline conditions were studied at pH of 8, 9, 10, and 11.

### 5.2.1 Effect of s/w Ratio on Alkaline Extraction of Oil and Proteins

In an earlier part of this investigation we used the s/w ratio of 1:3.5 which was suitable when extraction took place at neutral pH. However, the increase in the pH of extraction caused a large increase in viscosity of the slurry (Fig. 5). The high viscosity of the slurry made it hard to separate the slurry by centrifugation, thus, leaving a lot of oil in the solid residue. This in turn reduced the oil yield.

In this experiment the ground rapeseeds were enzyme-deactivated by dispersing them in boiling distilled water for two minutes at solid/water ratios of 1:3.5, 1:7, and 1:10. Every ratio was studied at pH 8, 9, 10, and 11. The desired pH values were adjusted before blending at 18,300 rpm for 10 minutes. The pH values were maintained throughout the experiment, including mixing at 2000 rpm for one hour at 70°C and centrifugation at 9000 g for 10 minutes.



The results, as shown in the Figure 11, indicated an increase in nitrogen solubility with the increase in pH in the alkaline direction. This was in agreement with earlier findings and with the findings of several workers regarding defatted meal (Radwan and Lu, 1976; Gillberg and Tornell, 1976; Yang *et al.*, 1978). The general trend indicated an increase in nitrogen solubility with the increase in amount of water added at pH 9, 10 and 11. However, no effect was found when extraction took place at pH 8, where the nitrogen solubility remained almost unchanged at about 35% at all used s/w ratios. The maximum nitrogen solubility was 81.5% at pH 11 and s/w ratio of 1:10.

Except with the decrease in oil yield at pH 10 and 11 and s/w ratio of 1:3.5 as a result of the high viscosity, the oil yield followed the same pattern as nitrogen (Fig. 12). This was because dissolving the protein material assisted in the release of oil into water dispersion (Sugarman, 1956).

The ratio 1:10 was selected to be used in further experiments since it gave the highest nitrogen solubility and the highest oil yield under the conditions of the experiment. A higher volume of solvent was not used because of the economic consideration. A higher volume of water would raise the percentage of oil and protein extracted. However, this increase in yield will coincide with an increase in the cost as a result of using more water and energy and problems associated with the disposal of a bigger



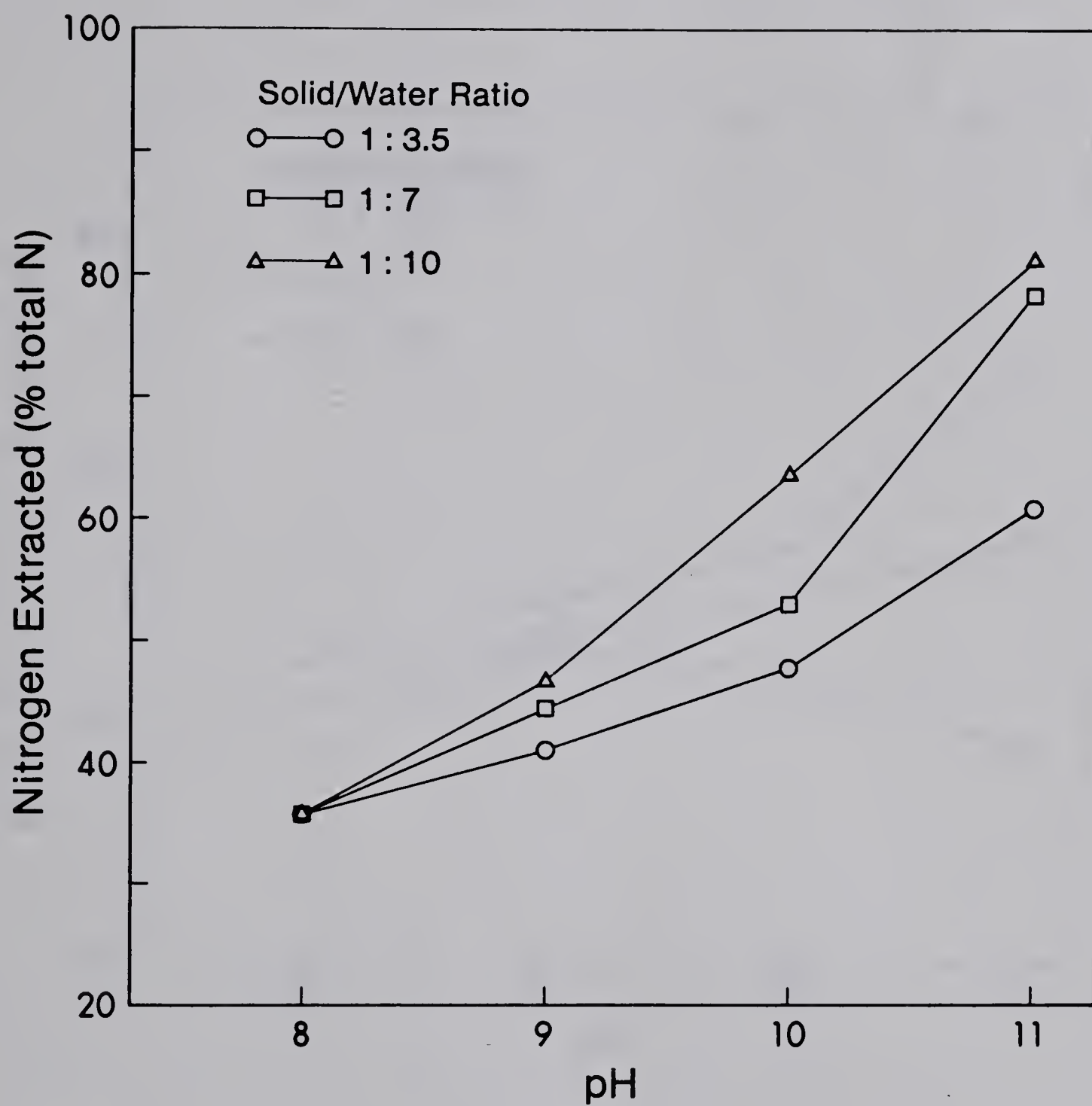


Fig. 11. Effect of solid/water ratio on nitrogen extractability from rapeseed slurries under alkaline conditions (blending 10 min, mixing 1 h at 70°C).





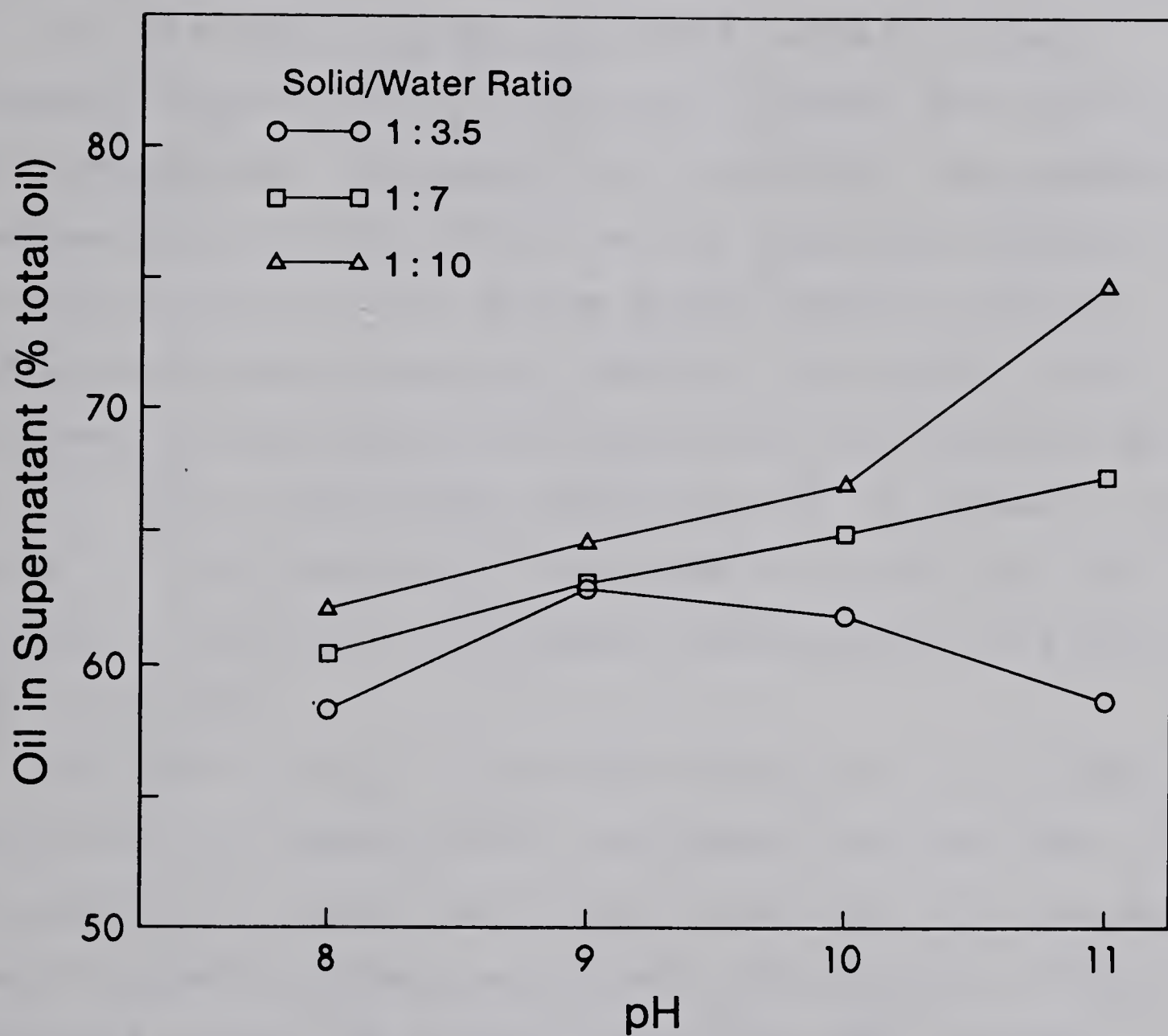


Fig. 12. Effect of solid/water ratio on oil extractability from rapeseed slurries under alkaline conditions (blending 10 min, mixing 1 h at 70°C).



volume of used water. However, if counter-current extraction was adopted, more water could be used in a given step.

### 5.2.2 Effect of Pre-extraction Heat Treatment on Alkaline Extraction of Oil and Protein

In this experiment the effects of wet and dry heat treatment applied during the process of enzyme deactivation was investigated. The yield of oil and protein from unheated seed was compared with yield from seed which was dispersed in boiling water for two minutes before cooling, and seed which was dry heat treated by industry. The effect of heat treatment on the yield of oil and protein was studied at pH 8, 9, 10 and 11. Extraction conditions were as follows: s/w ratio of 1:10, blending for 10 minutes at 18,300 rpm, and mixing for one hour at 70°C before centrifugation at 9,000 g for 10 minutes.

As shown in Fig. 13 the effect of dry heat on nitrogen solubility was insignificant. The results confirmed that it is possible to retain high nitrogen solubility after the dry heat treatment (Gillberg and Tornell, 1976; Girault, 1973; Rutkowski, 1970). The results showed also that nitrogen solubility was seriously impaired after the wet-heat treatment and extraction at pH 8 and 9. The effect was somewhat less at pH 10. At pH 11 almost no effect was observed; nitrogen solubilities were 84.1% and 81.5% for untreated and wet-heat treated seeds respectively. The difference was less than 3% compared with a reduction of



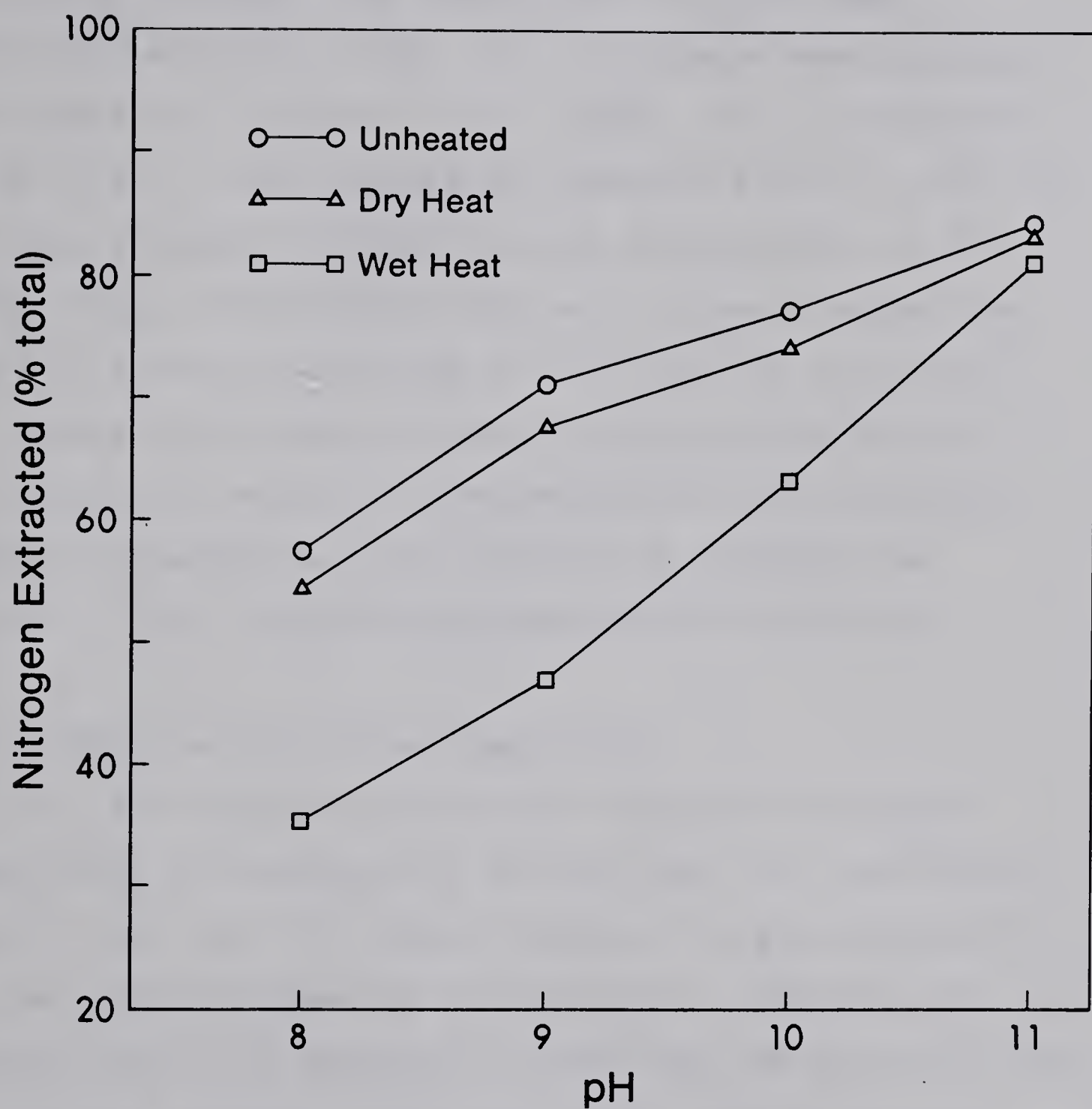


Fig. 13. Effect of pre-extraction heat treatment on nitrogen extractability under alkaline conditions. (s/w ratio 1:10, blending 10 min, mixing 1 h at 70°C)





more than 22% in nitrogen solubility at pH 8.

The effect of different methods of heat treatment on oil yield followed the same general pattern as the nitrogen solubility. However, the effect was not of the same magnitude. As shown in Fig. 14, the unheated seed produced the highest oil yield at all pH levels, with a maximum of 77.8% at pH 11 and a minimum oil yield of 65.5% at pH 8. The dry-heat treatment appeared to have little effect on oil yield at any pH; meanwhile, the wet-treatment reduced the yield by as much as 6% at pH 10 but less than 3% at pH 11.

Based on the above results, we selected to use pH 11 for the alkali extraction step because of the high protein yield. This agrees with the findings of Gillberg and Tornell, (1976) regarding maximum protein solubility.

### 5.2.3 Effect of Extraction Temperature

In this experiment the ground rapeseed was enzyme deactivated by dispersion in boiling water for two minutes, the s/w ratio was 1/10. After cooling, the pH was adjusted to the required values (8, 9, 10, and 11). Blending was carried out for 10 minutes at 18,300 rpm, and the mixing for one hour at a speed of 2000 rpm. Three temperatures of mixing were studied: 25, 50, and 70°C. We finished with twelve different mixing conditions by using three different mixing temperatures for each of the pH 8, 9, 10, and 11. The pH levels of the mixtures were maintained constant during mixing. Sodium hydroxide solution (20%) was added when



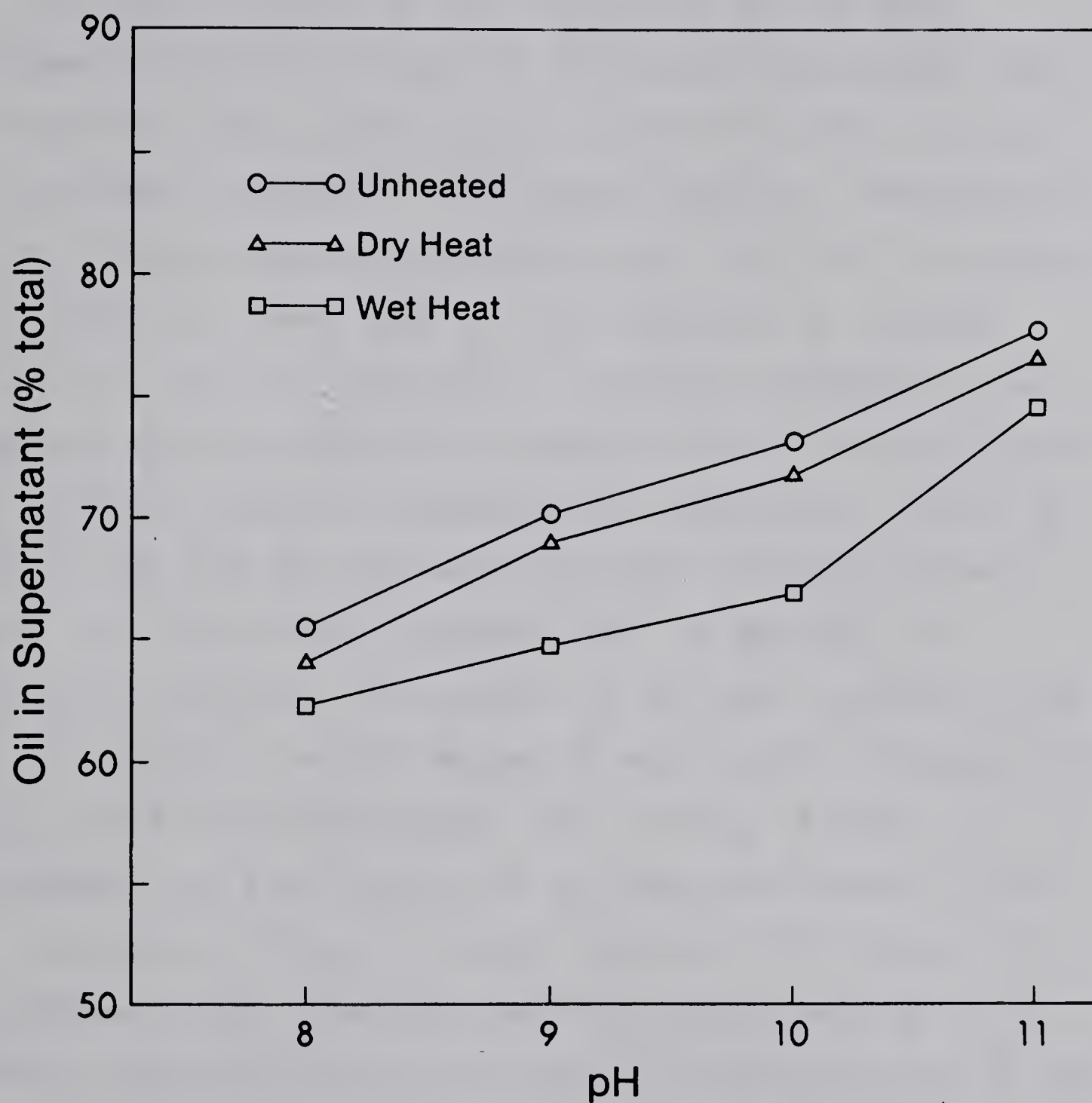


Fig. 14. Effect of pre-extraction heat treatment on oil extractability under alkaline conditions. (s/w ratio 1:10, blending 10 min, mixing 1 h at 70°C)



required. The amount of sodium hydroxide used for each batch was recorded and amount of sodium hydroxide was calculated as m mols/gm rapeseed.

As shown in Fig. 15, the solubility of nitrogen increased with the increase in extraction temperature. The nitrogen was most soluble at pH 11 and 70°C with 81.6% of the nitrogen extracted in the liquid fraction. Compared with 64.0% of the nitrogen extracted at pH 11 and 25°C this shows a difference of more than 17%. The increase in nitrogen solubility with the increase in extraction temperature was moderate when the extraction temperature was increased from 25° to 50°C. However, increasing the temperature from 50 to 70°C at the same pH increased nitrogen solubility by more than 12%. These results suggest that the decrease in nitrogen solubility, as a result of wet-heat treatment, can be overcome to a certain degree by working the extraction at high pH and high temperature. This finding is again in agreement with the findings of Gillberg and Tornell (1976).

As shown in Fig. 16 the oil yield at 25°C and pH 8, 9, and 10 was higher than the yield at temperatures of 50 or 70°C at those pH levels. Oil yield at temperatures of 50 and 70°C showed no appreciable differences between pH 8 and 11. The oil yields at pH 11 and temperatures of 25, 50 and 70°C were very similar. The increase in yield at the lower temperature might have been due to the fact that the emulsion, formed between the solubilized protein and the oil, is more stable at lower temperatures. Meanwhile, at





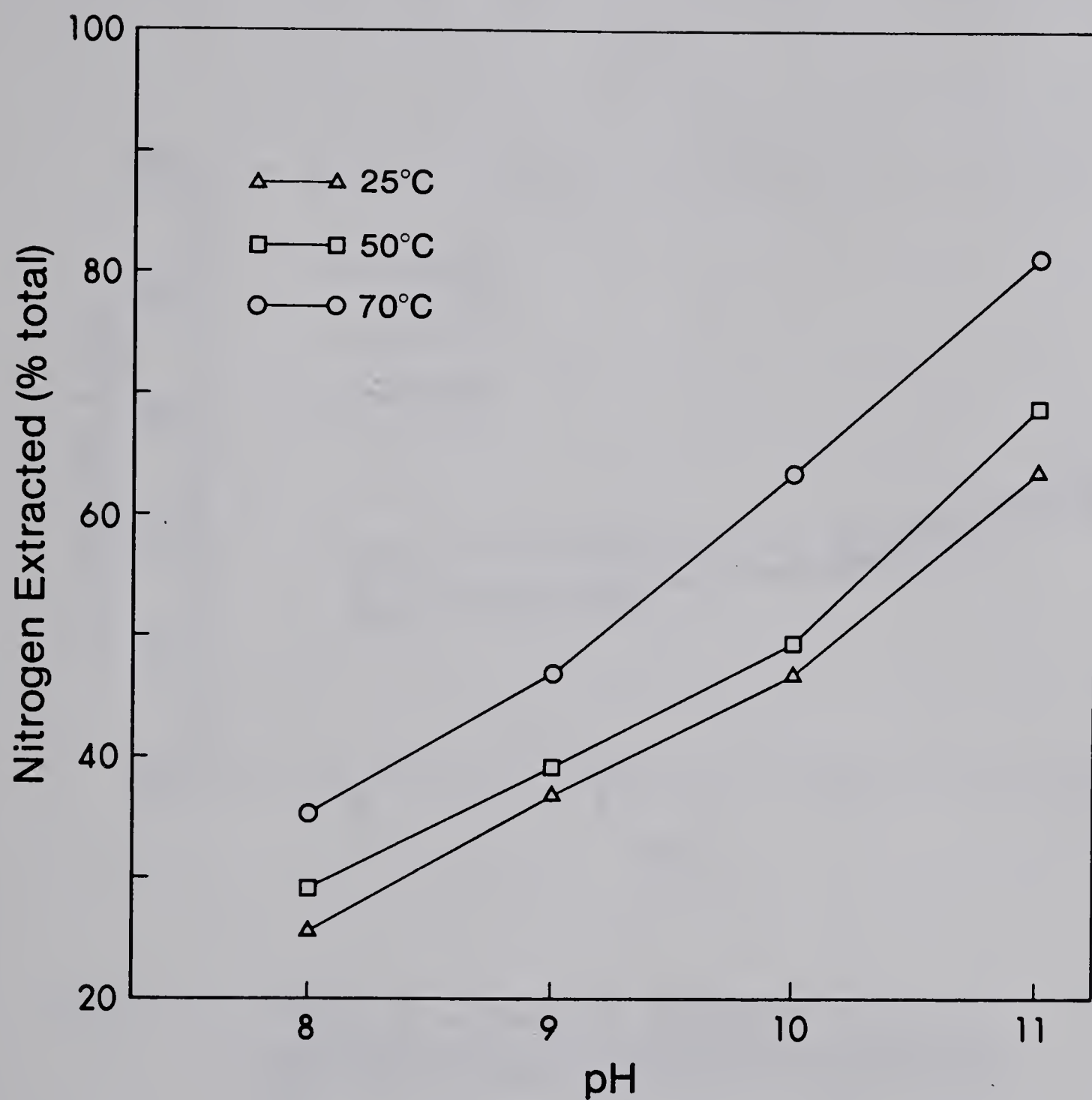


Fig. 15. Effect of extraction temperature on nitrogen extractability under alkaline conditions. (s/w ratio 1:10, blending for 10 min, mixing 1 h)



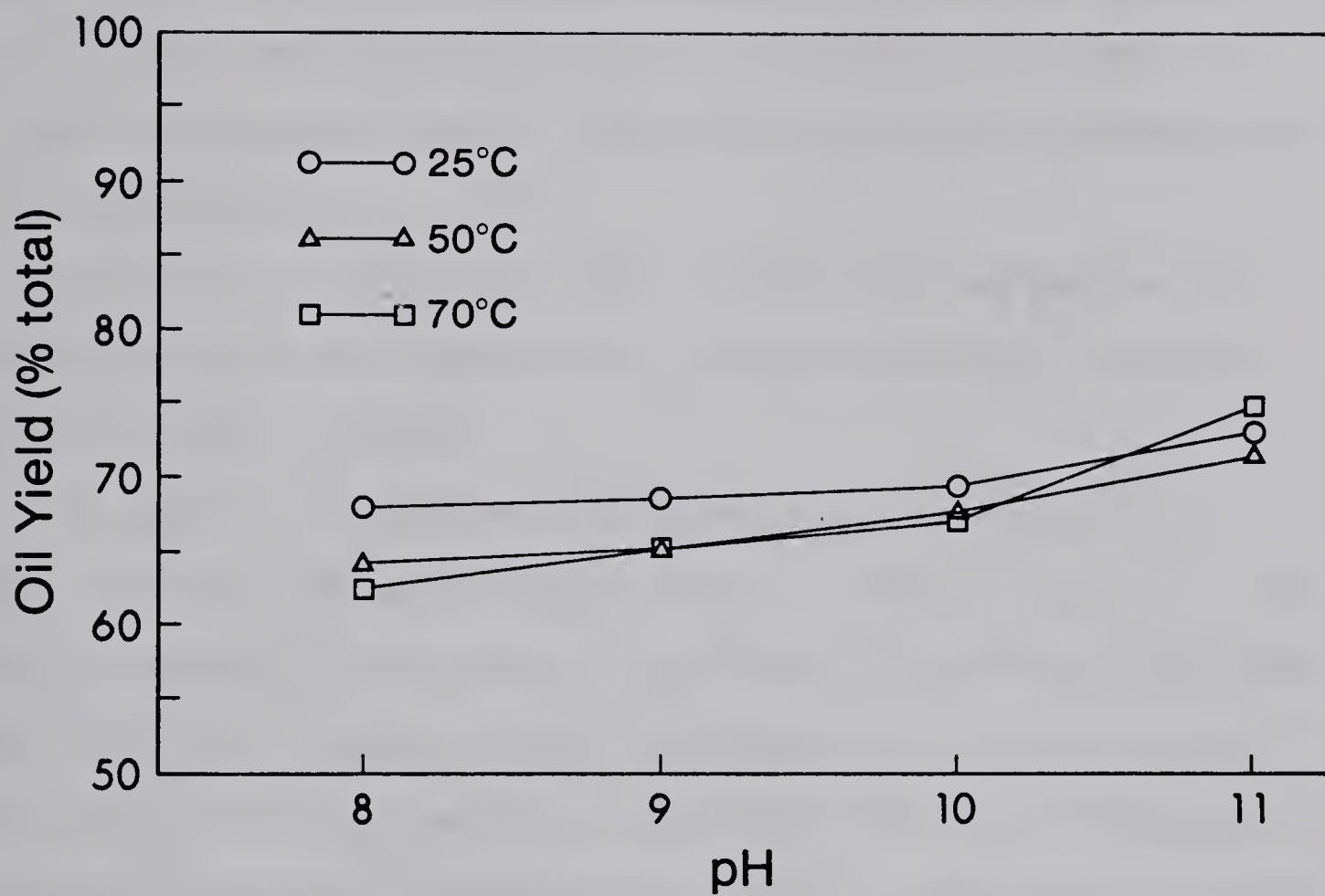


Fig. 16. Effect of extraction temperature on oil extractability under alkaline conditions. (s/w ratio 1:10, blending 10 min, mixing 1 h)



higher extraction temperatures, the heat destabilized the emulsion releasing some of the oil free. This oil might stay in the meal, thus reducing the oil yield. Heat treatment was used by many workers to break the emulsion. Rajasekharan and Sreenivasan (1976) separated coconut oil and protein from an aqueous of kernel by heating and centrifugation. Hagenmaier *et al.* (1972) and Hagenmaier (1977) subjected the emulsion of coconut cream to heat of 80-90°C before it was subjected to a shear force.

The high oil yield at lower temperatures might be also due to the combined effects of protein swelling, viscosity and centrifugal forces.

The amount of sodium hydroxide used to reach the required pH at the used temperature is shown in Fig. 17. The results showed an increase in the amount of sodium hydroxide used with the increase in pH and temperature of extraction. The increase in the amount of used NaOH with the increase in temperature was more pronounced at pH 11; the amount of NaOH was increased from 0.64 m mole/gm rapeseed at 25° to 1.20 m mol/gm at 70°C.

Although the extraction at higher temperatures increased the nitrogen solubility, thus increasing protein yield, it might have some adverse effect in the quality of protein and oil. In addition, extraction at 25°C produced a high oil yield. A decision on the temperature to be used for the extraction under alkaline conditions was delayed, until the results of tests related to protein and oil quality were





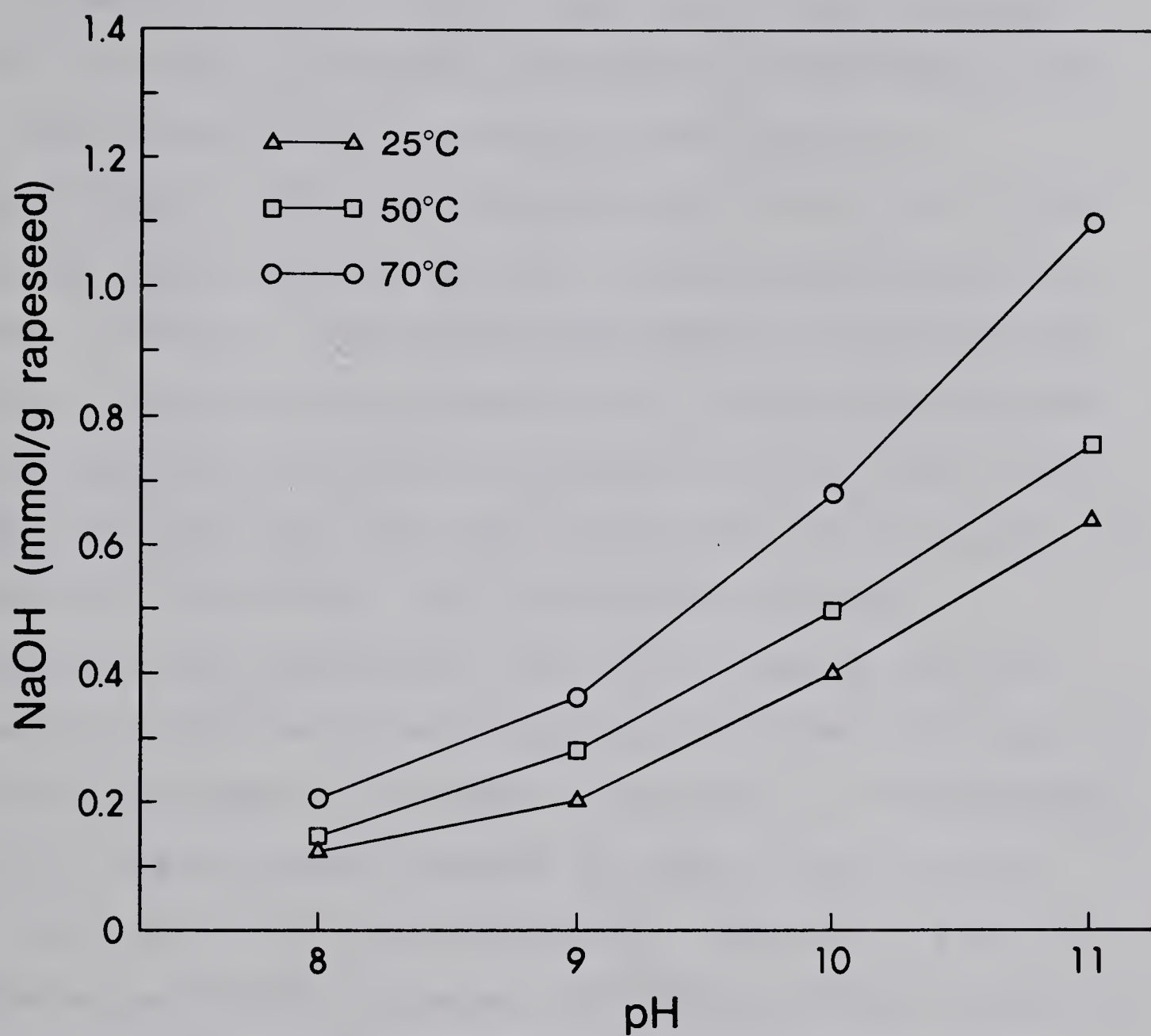


Fig. 17. Amount of sodium hydroxide used for pH adjustment of rapeseed slurries at different temperatures. (s/w ratio 1:10, blending 10 min, mixing 1 h)



available.

#### 5.2.4 Effect of Mixing Time on Alkaline Extraction of Oil and Protein

Conditions of extraction were as follows: s/w ratio 1/10; blending 10 minutes at a speed of 18,300 rpm and pH 11. The mixing was carried out at 2000 rpm, pH 11, temperature of 70°C. Mixing times were 15, 30, 45, and 60 minutes. Centrifugation was for 10 minutes at 9000 g. As shown in Table 9, under these experimental conditions the percent of nitrogen extracted in the liquid fraction after 15 minutes was 74.9% which increased to 79.8% after 30 min and 81.6% after one hour. This indicated that mixing time in excess of half an hour did not increase nitrogen extractability appreciably. This is in accords with the findings of Rutkowski and Korolczuk (1974) who even reported a slight decrease in nitrogen extractability with prolonged mixing. The oil yield followed the same pattern as the nitrogen solubility; no considerable increase in yield was found after 30 min of mixing. According to these results, it was decided to use 30 minute mixing periods in further experiments. A 30 minute mixing period was also used by other workers (Quinn and Jones, 1976; Yang al., 1978; Rutkowski and Korolczuk, 1974).



Table 9. Effect of mixing time on the extractability of oil and protein in solution under alkaline conditions\*

Extraction time (min)	15	30	45	60
Protein extracted (%total)	74.9a	79.8b	80.5b	81.6b
Oil extracted (%total)	69.4c	72.0c,d	74.1d	74.9d

\*Means of three readings. Figures in the same row not followed by the same superscript are significantly different ( $P \leq 0.05$ )





### 5.3 EFFECT OF ALKALI AND HEAT TREATMENT ON RAPESEED CONSTITUENTS

The exposure of rapeseed to high pH and high temperatures will result in changes in some components of rapeseed, particularly protein. Of special interest may be the effect of high heat and alkali treatment on the possible formation of new unusual amino acids such as lysinoalanine.

In addition, the extraction of rapeseed at elevated pH and temperature may raise concerns over formation of free fatty acids, and the possible darkening of protein (Sosulski and Bakal, 1969). This above treatment may also affect the stability of the harmful glucosinolates.

The quality of the final product of any process will determine the success or the failure of this process. A substantial deterioration in the nutritional value of the product coupled with the toxicological factors would make the final product nutritionally worthless.

For these reasons, a study of the effects of alkali and heat treatment on the various important constituents of rapeseed was deemed to be very important. In order to study the effects of heat and alkali treatment on rapeseed constituents, twelve combinations of heat (25°C, 50°C and 70°C) and alkali treatment (pH 8,9,10 and 11) were investigated. All the combinations were prepared in duplicate, and all analyses were performed on all samples at least twice. The indicators studied in the treated meals included the glucosinolates contents, lysinoalanine,



electrophoresis pattern, amino acid composition and color. The oil was tested for free fatty acids.

### 5.3.1 Effect of Alkali and Heat Treatment on the Free Fatty Acid Content of Oil

Table 10 shows the free fatty acid content of industrial crude oil, soxhlet extracted oil from untreated seed, oil extracted using the aqueous process and the oils extracted by soxhlet from rapeseeds which were exposed to different heat and alkaline treatments. The results indicate that oils extracted from rapeseeds treated at pH 8 and 9 at all temperatures and pH10 at 25 and 50°C did not show any appreciable increase in their free fatty acid content when they were compared with the industrial oil or soxhlet extracted oil from untreated rapeseed.

Oil extracted from rapeseed treated at pH 10 and 70°C, and pH 11 at 25 and 50°C, showed a slight increase in free fatty acid content to 0.6%. However, oil extracted from seed treatment at pH 11 and 70°C showed a sharp increase in free fatty acid content to as high as 2.05%. This sharp increase in free fatty acid content is undoubtedly due to fat hydrolysis which is the result of the combined effect of the high pH, high temperature, and the large amount of NaOH used to maintain the required high pH during mixing. As shown earlier in Fig 17, the amount of NaOH used to maintain the required pH 11 during extraction almost doubled when the temperature of extraction was increased from 25 to 70°C.



Table 10. Effect of alkaline extraction conditions on free fatty acid content of rapeseed oil

Sample and treatment		* Percent of free fatty acid
-industrial crude oil		0.51-0.55
-soxhlet extracted crude oil (no treatment)		0.48-0.50
-oil extracted using aqueous process at natural pH		0.44-0.50
-soxhlet extracted oil from rapeseed treated at:		
pH	Temperature C	
8	25	0.48-0.52
8	50	0.48-0.50
8	70	0.51-0.55
9	25	0.45-0.50
9	50	0.49-0.55
9	70	0.49-0.53
10	25	0.47-0.53
10	50	0.50-0.54
10	70	0.56-0.60
11	25	0.58-0.60
11	50	0.57-0.60
11	70	1.95-2.05

\*Maximum and minimum of four analyses







Amounts of free fatty acids in industrial rapeseed crude oil reported in the literature are 0.55% (Embong, 1977), 0.4 to 1.5% (Teasdale, 1975), 0.5% (Pritchard, 1974), 0.97% (Hoffman, 1973), and 0.3 to 0.5% (Appelqvist, 1961). Rhee *et al.* (1973) found that free fatty acid content of peanut oil increased slightly with increasing extraction pH. Meanwhile, Hagenmaier (1974) found no change in free fatty acid content of oil from sunflower seeds when they were incubated for one hour at temperatures ranging from 5-65°C and pH from 4.5 to 10. These results agree with ours.

The free fatty acids are usually removed from the oil during the refining process in order to stop any off-flavor developing in the oil. The removal of free fatty acids is enhanced during refining by employing alkali or steam, and they are also removed during the deodorization process (Anjou, 1972). According to Moysey and Norum (1975), high content of free fatty acids represents high losses of oil during processing. Losses of industrial extracted crude oil during refining are about 0.8% or less when good quality seed is used (Downey *et al.*, 1974). According to Olin (1957), the amount of free fatty acids in crude oil of good quality should not exceed 1.5% in order to avoid high refining losses.

The only alkali treatment which produced oil with high free fatty acids content was the treatment of pH 11 and 70°C. Other treatments produced good quality oil with maximum free fatty acids content of 0.6%. Accordingly, in



order to produce good quality oil, high extraction temperatures at pH 11 or higher should be avoided.

Rapeseed, unlike most other oilseeds, contains high amount of sulphur. The sulphur source are the glucosinolates, which upon hydrolysis release various sulphur compounds. These are soluble in oil and could poison the catalyst during the hydrogenation process (Sosulski, 1974). Working on the aqueous extraction of rapeseed, Embong (1977) investigated the effect of pH during stirring on the sulphur content of the oil. He found virtually no effect of pH on sulphur content in the oil. We found no need to repeat his work.

### 5.3.2 Effect of Alkali and Heat Treatment on Color of Meal and Meal Fractions

The color of a product will help to determine its usefulness. A product with light color can be used in a wide variety of products, while dark color seriously limits the potential uses of rapeseed protein.

In our study, color was determined for (a) freeze-dried, defatted, rapeseed samples which were neutralized to the original pH (5.8) after exposure to the different heat and alkaline treatment, and (b) for the liquid fractions resulting from the centrifugation of the same heat-alkali treated rapeseed samples neutralized after centrifugation and before freeze-drying and defatting. Figures 18 and 19 illustrate color changes caused by the





alkali treatment of defatted rapeseed samples. The untreated and the industrial meals were used as a reference for comparison. The color of the industrial rapeseed meal was still darker than our meal which was exposed to the severest treatment (sample 14, treated at pH 11 and 70°C for one hour). The color of the meals was getting darker with the increase in pH and temperature. In particular the sample #2 which was not exposed to alkali, might suggest that the effect of the temperature is more important than pH in producing the dark color. This is well illustrated in samples 3, 4, 5 and 6 which were treated at pH 8, 9, 10 and 11, respectively, and 25°C, when they are compared with samples 11, 12, 13 and 14 which were treated at the same pH but at 70°C.

Along with the photographic illustration, the color differences among the aforementioned samples were substantiated by objective measurement using the Hunterlab colorimeter. The L value (lightness) was deemed to be the most important and was used as an indicator of the color of the different meals as shown in Fig. 19. The L value for untreated meal was 77.3 compared with 40.1 for industrial meal and 54.1 for our most severe treatment (pH at 70°C for one hour).

The color of the neutralized freeze dried liquid fractions was also compared using the Hunter colorimeter (Table 11). The L values for the liquid fractions followed the same pattern as those of the whole meal. Photo in Fig.







Fig. 18. Effect of alkaline-heat treatment on color of rapeseed meal.

1. Soxhlet-extracted untreated rapeseed.
2. Industrial rapeseed meal.
- Soxhlet-extracted seed after the following treatments:
3. pH 8 at 25°C
4. pH 9 at 25°C
5. pH 10 at 25°C
6. pH 11 at 25°C
7. pH 8 at 50°C
8. pH 9 at 50°C
9. pH 10 at 50°C
10. pH 11 at 50°C
11. pH 8 at 70°C
12. pH 9 at 70°C
13. pH 10 at 70°C
14. pH 11 at 70°C



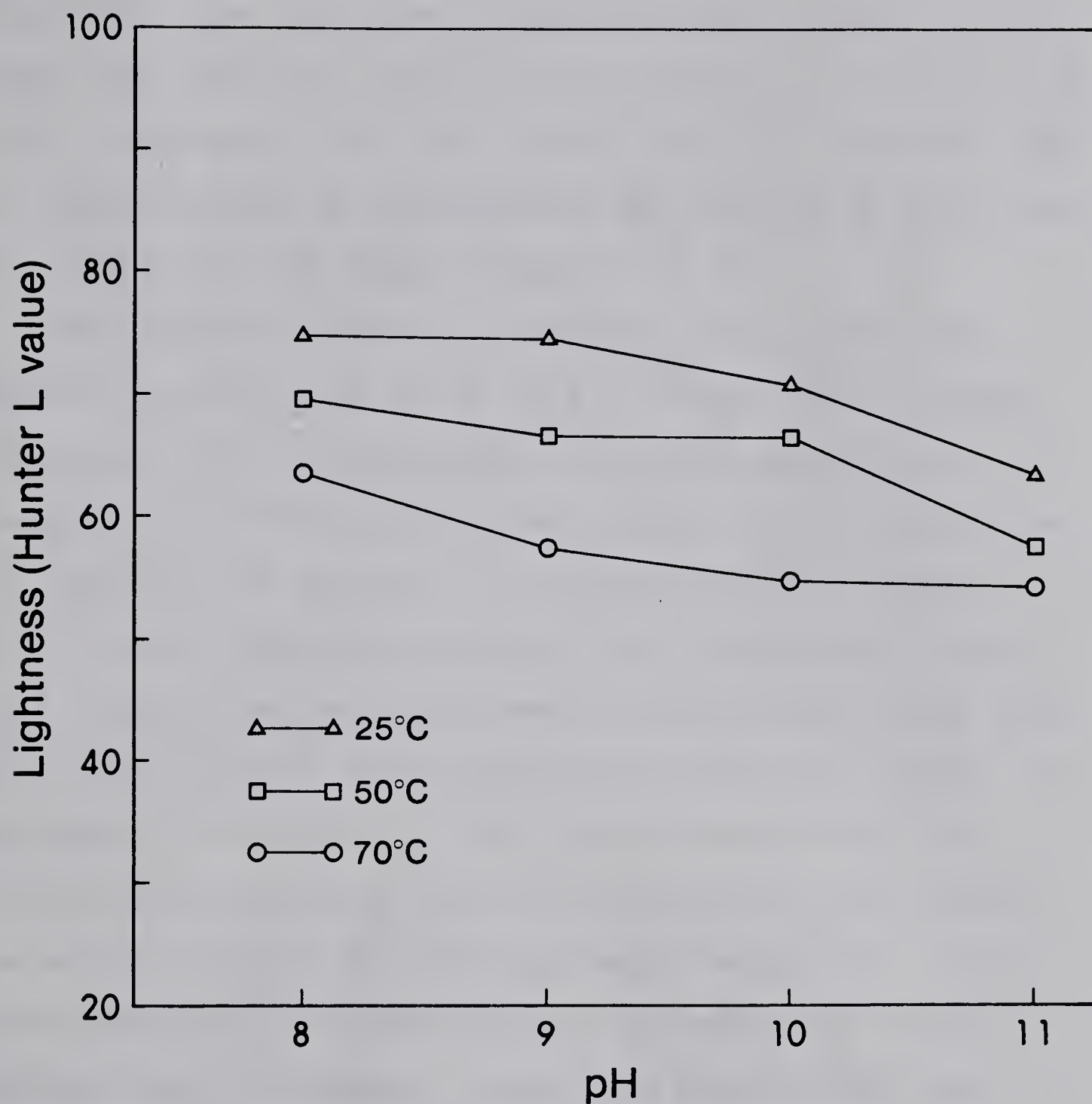


Fig. 19. Effect of different alkali extraction conditions on the color of rapeseed meal. L = 100 (white), L = 0 (black)  
L values for untreated and industrial meals were 77.3 and 40.1, respectively.



20 also illustrates this point. Heating the rapeseed slurry at the natural pH (5.8) and 80°C for ten minutes produced a liquid fraction with an average L value of 71.9. When the mixing time under the above conditions was increased to 30 minutes the resulting liquid fraction had an average L value of 62.7, suggesting that this liquid fraction is darker than the liquid fraction extracted from seed treated at pH 11 and 25°C for one hour (average L value of 64.2).

These results confirm an earlier finding about the temperature playing the major role in discoloration. Table 11 also shows that when rapeseed was first extracted at the natural pH at 80°C for 10 minutes before it was extracted at pH 11 and 25°C for one hour, the resulting liquid fraction had an L value which was slightly higher than the L values of the liquid fractions from seed treated at pH 11 and 25°C for one hour without going through the first step. Thus, an improvement in the color of the liquid fraction may be achieved by employing an initial extraction of the meal by water at the natural pH. The improvement might be a result of the removal in the first step of low-molecular weight compounds such as pigments, sugars and amino acids that react to form melanoidins imparting a dark brown color to the meal as well as to the aqueous extract (Rutkowski, 1970).

During neutralization, the alkaline aqueous fractions acquired different colors. This was also noticed by Yang *et al.* (1978) who found the precipitate to have a light cream





Table 11. Hunterlab colorimeter readings for aqueous fractions two batches of alkali treated rapeseed samples.

	Sample and Treatment			L Value*	
	pH	Temperature (C°)	Time (min)	Batch 1	Batch 2
1	8	25°C	60	75.8	72.6
2	9	25	60	69.1	71.5
3	10	25	60	63.8	66.0
4	11	25	60	64	64.6
5	8	50	60	68.6	65.8
6	9	50	60	64.6	63.8
7	10	50	60	59.8	61.4
8	11	50	60	61.0	59.6
9	8	70	60	63.0	65.8
10	9	70	60	62.3	61.5
11	10	70	60	53.9	56.9
12	11	70	60	54.3	55.5
13	5.8(natural)	80	30 min	64.2	61.6
14a	5.8(natural)	80	10 min	69.9	73.9
15b	11.0	25	60	65.9	68.3
16c	-	-	-	83.7	84.9

\* Average of three virtually identical replicate readings within each batch.

(a) First extraction at natural pH.

(b) Second extraction of meal produced from the above first extraction.

(c) Rapeseed flour (dehulled defatted)



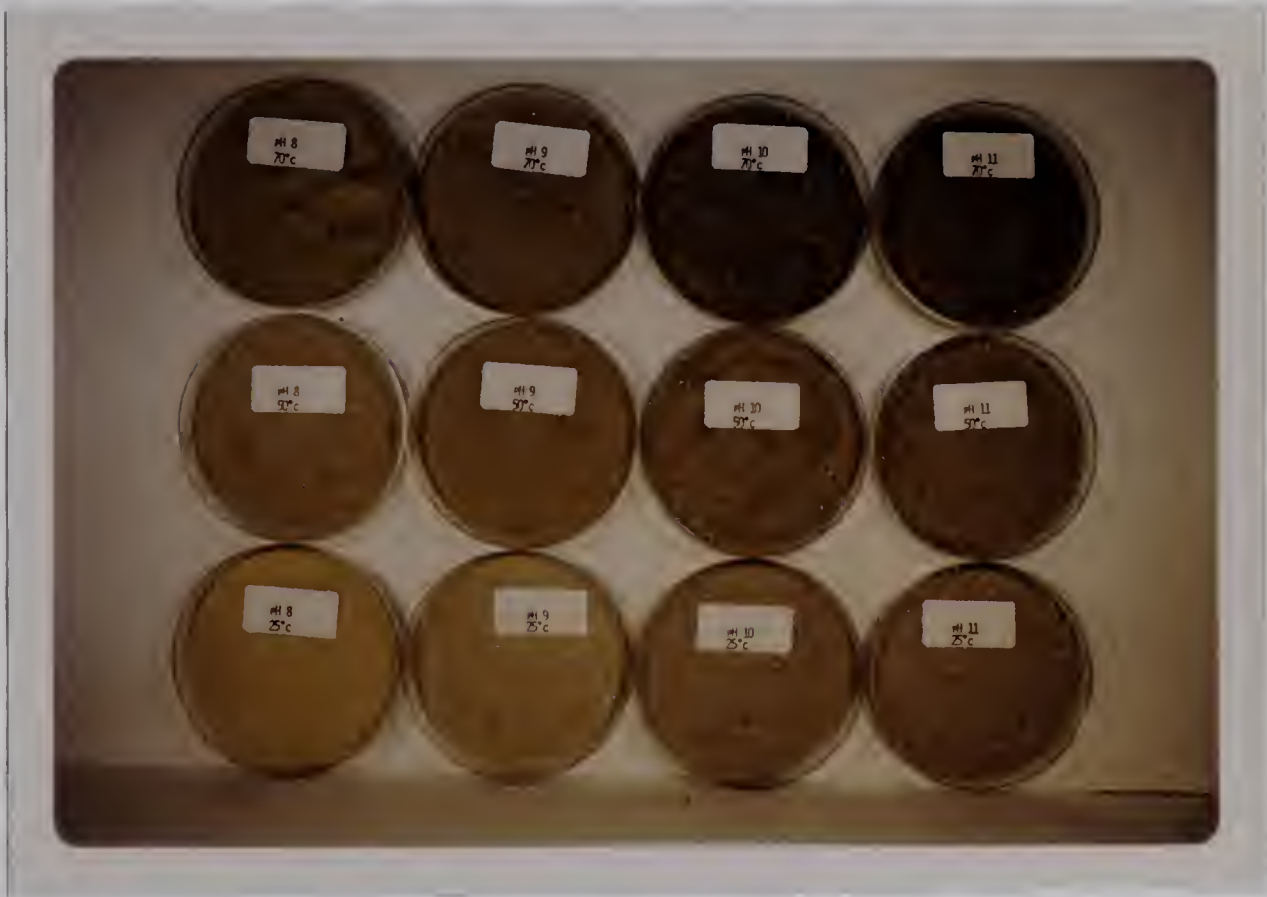


Fig. 20. Effect of alkaline extraction conditions on the colors of the defatted freeze-dried liquid fractions.



color at pH 5.6, yellow-cream at pH 5, and gray at pH 6.7. Their extract was prepared at pH 11 and 4°C, thus avoiding the high temperature conducive to the production of a darker color.

The above results suggest that in order to reduce the discoloration, extraction should be conducted at low temperatures and short times. Washing as the first step was also found to produce a product with lighter color (Table 11).

### 5.3.3 Effect of Alkali and Heat Treatment on Glucosinolates Content

The existence of glucosinolates in the rapeseed limits the use of rapeseed protein for human and animal consumption. Many processes have been developed to reduce or eliminate the potentially toxic glucosinolates. In our first step, extraction was expected to remove high proportion of the glucosinolates along with the oil.

The defatted rapeseed samples exposed to different heat-alkali treatments from the previous experiment were analyzed for the total glucosinolates content in the meal as well as in liquid and solid fractions of the treated rapeseed slurries. The purpose of this investigation was to study the effect of the different alkali-heat treatments on the glucosinolates content of the defatted meals, and also the distribution of the remaining glucosinolates in both the liquid and the solid fractions. The results shown in





Table 12 indicate a reduction in the glucosinolates content in all treated samples as compared to untreated defatted rapeseed. At the most severe treatment (pH 11 at 70°C) all of the glucosinolates disappeared. At pH 10 and 70°C the percentage of remaining glucosinolates was 20.2%.

The results indicated that in order to eliminate all the glucosinolates the rapeseed has to be treated at least at pH 11 at 70°C. The total glucosinolates content of the rapeseed before and after treatment was determined by the McGregor method. The method involves hydrolysis of the glucosinolates using the enzyme myrosinase and then measuring the hydrolysis products as the thiourea derivative of the isothiocyanates and/or oxazolidinethiones.

When the above method was employed with all of the treated samples but without adding the enzyme myrosinase, no glucosinolates hydrolysis products were detected, thus indicating that the enzyme deactivation treatment was efficient and the degradation products do not include the toxic substances of isothiocyanates and/or oxazolidinethiones.

During the myrosinase deactivation process, other more heat labile factors which are involved in nitriles formation upon enzymatic hydrolysis of the glucosinolates will also be deactivated. Nitriles are usually formed after the enzymatic hydrolysis of glucosinolates by myrosinase producing various unstable chemical compounds which are transformed to isothiocyanates and nitriles (Bjorkman, 1976). This means



Table 12. Effect of heat and alkali treatment \* on the disappearance of glucosinolates from defatted rapeseed meal.

No	pH	Temperature (°C)	Glucosinolates content (mg/g)**		% of remaining *** glucosinolates
			Mean	St. dev.	
1	11	70	0.00	(0.00)	00.0
2	10	70	0.58	(0.07)	20.2
3	9	70	1.13	(0.12)	39.4
4	8	70	1.37	(0.23)	41.3
5	11	50	0.79	(0.08)	27.5
6	10	50	1.11	(0.25)	38.7
7	9	50	1.14	(0.20)	39.7
8	8	50	1.13	(0.15)	39.4
9	11	25	1.09	(0.19)	36.2
10	10	25	1.04	(0.30)	38.0
11	9	25	1.13	(0.13)	39.4
12	8	25	1.30	(0.20)	46.4

\*. All the samples were treated for one hour

\*\*Means and (st. dev) of duplicate determination of two batches.

\*\*\*. As compared to the original amount in a control sample (untreated meal).



that in the absence of the enzyme myrosinase no nitriles will be formed; also, all our treatments were under alkaline conditions, while nitriles formation is favored at a low pH (Van Etten *et al.*, 1966).

No further analyses were conducted to characterize or to determine the safety of the degradation products. However, the results indicated that the degraded products do not include the aforementioned harmful enzymatic hydrolysis products.

Degradation of glucosinolates, as a result of heat treatment, was reported by several workers (i.e. Josefsson, 1975). Kozłowska *et al.*, (1974) mentioned that toasting inactivated the goitrogenic agents probably by the transformation of 5-vinyl-1,3-oxazolidin-2-thione to 5-vinyl-1,3-thiazolidinone.

Table 13 shows the total glucosinolates content of both liquid and solid fractions obtained from alkali-heat treated rapeseed samples. The results indicate that the maximum amount of glucosinolates remaining in the solid fraction was about 0.13 mg/gm sample at the most moderate alkali-heat treatment (pH 8 and 9 at 25°C).

In our opinion, this maximum is low if we compare it with that of the glucosinolates content of cabbage which was found to be as high as 1.105 mg/gm of fresh cabbage (Daxenbichler *et al.*, 1980). The table also shows that in all treatments, most of the glucosinolates were leached into the liquid fractions. At 70°C the solid fractions at pH 11,







Table 13. Total glucosinolates content of solid and liquid extracts of alkali-heat treated rapeseed.

No	Treatment*		Glucosinolates content** as mg 3-butenyl isothio-cyanate/gm sample	
	pH	Temperature (°C)	Liquid fraction	Solid fraction
1	11	70	0.00 (0.00)	0.00 (0.00)
2	10	70	0.40 (0.36)	0.00 (0.00)
3	9	70	1.06 (0.21)	0.00 (0.00)
4	8	70	1.00 (0.10)	0.03 (0.06)
5	11	50	1.31 (0.35)	0.10 (0.17)
6	10	50	0.63 (0.06)	0.06 (0.06)
7	9	50	1.03 (0.15)	0.03 (0.06)
8	8	50	1.03 (0.25)	0.10 (0.10)
9	11	25	1.66 (0.15)	0.10 (0.10)
10	10	25	0.86 (0.12)	0.06 (0.12)
11	9	25	0.73 (0.06)	0.13 (0.15)
12	8	25	1.46 (0.15)	0.13 (0.10)
13	5.8	80	1.60 (0.26)	0.06 (0.05)
14	untreated rapeseed meal (soxhlet extracted)		2.87 mg/gm	

\*All treatments were at one hour except sample #13 (30 min)

\*\*Means and (st. dev.) of triplicate determinations of two batches.



10 and 9 contained no glucosinolates, meanwhile at pH 8 it contained a mere 0.03 mg glucosinolates/gm of the sample. The above results suggest the alkaline-heat treatment does not only degrade the glucosinolates, but it may also enhance the leaching of glucosinolates into liquid fraction. This is in agreement with the finding of several workers (Sarwar *et al.*, 1975; Kozłowska *et al.*, 1974; Bhatti and Sosulski, 1972, Sosulski *et al.*, 1972).

Extraction at the natural pH (5.8) for 30 minutes at 80°C produced a meal with glucosinolates content of 0.066 mg/gm, which is very low. This suggests that a first extraction at a high temperature would remove most of the glucosinolates before the second step of alkaline extraction, thus helping to produce a better quality meal and protein concentrate.

#### 5.4 EFFECT OF ALKALI-HEAT TREATMENTS ON RAPESEED PROTEIN

This part of the study was undertaken to determine the effect of the different alkali-heat treatments on rapeseed protein and its nutritional value. The three indicators used were lysinoalanine, the amino acid profile and gel electrophoresis pattern.



#### 5.4.1 Lysinoalanine (LAL) formation

As shown in Table 14, four of the twelve treatments showed formation of lysinoalanine (LAL). The results indicate that heat treatment played an important role in the production of LAL since the LAL formation was the highest (2550 ppm) at pH 11 at 70°C. It dropped to 1060 ppm at pH 11 at 50°C, while no LAL was detected at pH 11 at 25°C.

The LAL content at pH 10 at 70°C (2493 ppm) and pH 11 at 70°C (2550 ppm) were virtually identical and much higher than that of pH 11 at 50°C (1060 ppm).

At pH 9 LAL formation was found only at 70°C. LAL was not detected at either 50 or 25°C. No LAL was detected for all pH values at 25°C or for pH 8 at all temperatures.

Our findings show that the treatment at high temperature and moderate alkali conditions caused the formation of LAL (pH 9 at 70°C), meanwhile a low temperature at a higher pH (pH 11 at 25°C) did not produce any LAL. This might suggest that in case of rapeseed protein, high temperature is required to produce LAL in alkali treated rapeseed protein. These results are in agreement with the findings of Sternberg *et al.* (1975,a,b,) regarding the importance of heat as factor in producing LAL. Yang *et al.* (1978), who extracted rapeseed protein at pH 11 and 4°C, observed no lysinoalanine formation. The fact that we found LAL at some of the treatments indicates that other compounds such as lanthionine, ornithinoalanine and alloisoeicine might have been formed along with LAL as a result of the





Table 14. Effect of alkali-heat treatments on the production of lysinoalanine (LAL) in defatted rapeseed meal\*

SAMPLE*	pH	Temperature (°C)	LAL(a) ppm.
1	11	70	2550
2	10	70	2493
3	9	70	680
4	8	70	not detected
5	11	50	1060
6	10	50	not detected
7	9	50	not detected
8	8	50	not detected
9	11	25	not detected
10	10	25	not detected
11	9	25	not detected
12	8	25	not detected
13b	5.8	80	not detected
14c	5.8	80	not detected
15d	11	25	not detected
16	commercial rapeseed meal		not detected

\* Average of at least two batches.

a. Treatment time is one hour unless otherwise indicated.

b. Extraction for 30 minutes.

c. First extraction at natural pH, for 10 minutes.

d. Second extraction of #14 for one hour.



heat-alkali treatments (Provansal *et al*, 1975). LAL, which is a much investigated compound, was found to be harmful to rats (Woodard and Short, 1973). However, feeding six other animal species with protein containing LAL at levels greatly in excess of that which is uniformly toxic to rats (100 ppm) did not induce the same harmful effect (O'Donovan, 1976). This suggests that the harmful effect of LAL maybe a phenomenon specific to the rat species. Also the findings of LAL in food which was not exposed to alkali treatment (Sternberg *et al* ., 1975a, b) indicate that man has been exposed to LAL for a long time. However, our literature survey has not yielded any solid proof that LAL or any other compound which might be produced as a result of the heat-alkali treatment is completely safe. The fact that the nutritional and toxicological consequences of the produced amino acid derivatives are not yet known suggests that to be safe, any treatment of rapeseed protein should avoid high heat with alkaline conditions. Accordingly, these results suggest the use of pH 11 at 25°C with the extraction period as short as possible.

#### **5.4.2 Amino Acid Profile of Rapeseed Proteins Exposed to Different Alkali-Heat Treatments**

The amino acid profiles of rapeseed proteins treated at the 12 selected combinations of pH and temperature are reported in Table 15. At most of the treatments there were no considerable changes in the amino acid profile. However,



Table 15 Amino acid composition of rapeseed protein after various heat and alkali treatments

sample	1	2	3	4	5	6	7	8	9	10	11	12	13
pH	-	11	10	9	8	11	10	9	8	11	10	9	8
Temperature	-	70°C	70°C	70°C	70°C	50°C	50°C	50°C	50°C	25°C	25°C	25°C	25°C
Duration	-	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr	1hr
ASP	7.37	7.41	7.32	7.45	7.41	7.25	7.36	7.34	7.29	7.46	7.43	7.21	7.26
THR*	4.67	4.51	4.62	4.61	4.59	4.56	4.67	4.59	4.36	4.61	4.64	4.59	4.73
SER	4.40	3.96	4.12	4.24	4.38	4.19	4.34	4.39	4.43	4.48	4.37	4.46	4.39
GLU	17.91	18.12	18.03	17.91	17.86	17.91	18.09	17.86	18.15	17.93	17.78	18.02	17.81
PRO	7.11	7.02	6.91	6.98	7.15	7.02	6.86	6.84	7.06	7.19	7.16	6.96	7.07
GLY	4.89	4.81	4.92	4.76	4.71	4.93	4.76	4.71	4.68	4.93	4.78	4.68	4.84
ALA	4.43	4.39	4.48	4.31	4.36	4.29	4.38	4.45	4.26	4.16	4.41	4.26	4.38
1/2 CYS	1.19	0.00	0.00	0.71	1.09	Traces	1.07	1.17	1.11	1.07	1.17	1.09	1.23
VAL*	5.15	5.18	5.27	5.06	5.11	4.98	5.16	5.28	4.94	4.98	5.08	4.96	5.11
MET*	1.85	1.81	1.78	1.73	1.82	1.71	1.69	1.84	1.64	1.72	1.79	1.66	1.79
ILU*	3.87	3.81	3.97	3.74	3.81	3.89	3.81	3.85	3.78	3.96	3.93	3.83	3.79
LEU*	6.90	6.92	6.77	6.83	6.89	7.07	6.78	6.90	7.01	6.74	6.97	6.81	6.92
TYR	2.61	2.54	2.59	2.49	2.68	2.59	2.71	2.51	2.51	2.64	2.72	2.51	2.63
PHE*	3.93	4.00	3.90	3.83	3.96	3.87	4.11	4.07	3.99	3.79	3.81	3.98	3.91
HIS*	2.65	2.70	2.61	2.64	2.58	2.54	2.72	2.78	2.61	2.54	2.67	2.60	2.71
LYS*	5.71	4.78	4.89	4.93	5.37	5.27	5.56	5.48	5.41	5.53	5.61	5.53	5.56
ARG*	5.89	5.32	5.47	5.54	5.88	5.69	5.73	5.71	5.78	5.80	5.83	5.01	5.78

\*Essential amino acids  
-Amino acid affected







the samples which showed lysinoalanine formation showed also some amino acids changes. The amino acids most affected are underlined in the Table 15. Cystine was found to be the most affected amino acid since it disappeared completely at pH 11 and 10 at 70°C and was greatly reduced at pH 9 at 70°C; only traces were found at pH 11 and 50°C. Little change was found at the remaining treatments. Decreases in the content of the amino acids arginine, lysine, serine, and threonine were also found to occur as indicated in the Table 15. The above results are in accord with results of other investigators with different proteins (Provansal *et al.*, 1975; De Groot and Slump, 1969, Friedman, 1979). Isomerization of amino acids was not investigated in this work. However, isomerization has been found to occur through alkaline treatment (Hill and Leach, 1964, Pollock and Frommhagen, 1968). Destruction of amino acid residues, formation of new cross-links and/or isomerization of amino acids were found to lower the nutritional values of the proteins (i.e. Provansal *et al.*, 1975, De Groot and Slump, 1969, Friedman, 1979).

#### 5.4.3 Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis Pattern of Alkali-Heat Treated Rapeseed Proteins

Polyacrylamide gel electrophoresis in the presence of SDS separates the polypeptide chains according to their molecular weights. Thus, the molecular weights of the



polypeptide chains of a given protein can be determined by comparing their electrophoretic mobilities on SDS gels to the mobilities of marker proteins with polypeptide chains of known molecular weights.

The patterns shown in the Fig. 21 may suggest that between the twelve treatments applied, there were only three treatments which caused a change in the electrophoretic pattern. These samples were 3,4 and, to a lesser degree, 5. The most noticeable changes were in samples 3 and 4 which were treated at pH 11 and 10 at 70°C. The changes in the pattern might indicate hydrolysis of the large molecular weight proteins to smaller ones, or to small peptides or amino acids which do not appear in the gel. The changes in pattern may possibly result in the formation of complicated compounds between the protein and other rapeseed constituents. The newly formed compounds might not penetrate the gel. Evidence for such large compounds maybe seen at the surface of gels 3, 4 and 5 in Fig. 21. Further studies are needed to determine the reason or reasons for the changes in the pattern.

The possible hydrolysis of proteins by alkali-heat treatments is expected to cause racemization of amino acids resulting in a reduction in the nutritional value of the proteins (Anglemier and Montgomery, 1976). Hydrolysis also will cause a reduction in protein yield because small peptides and amino acids can not be recovered by precipitation.





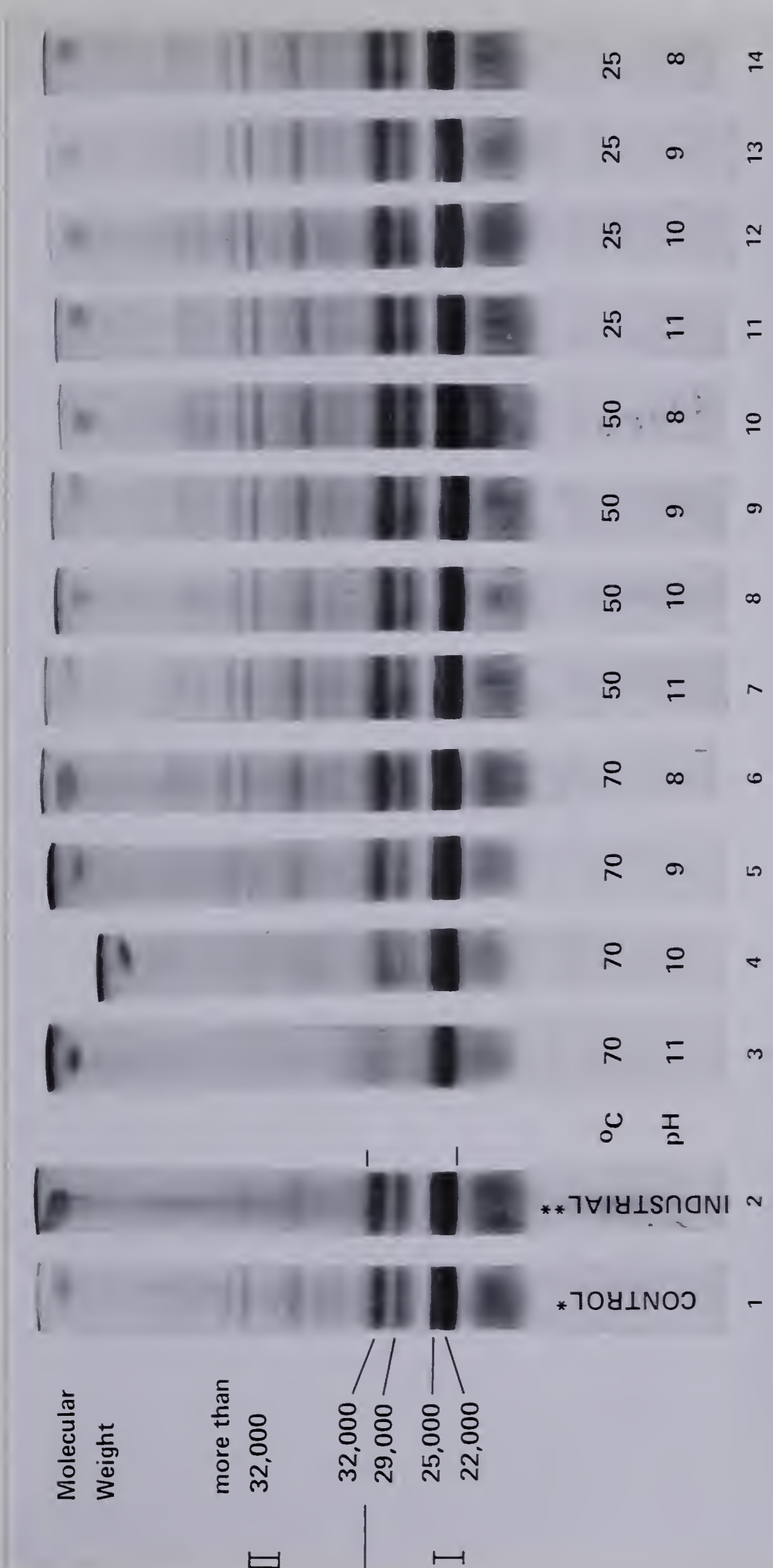


Fig. 21. Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis pattern of alkali heat treated rapeseed proteins.





Our investigation of the effect of alkali-heat treatments on the rapeseed protein indicated that severe alkali-heat conditions would produce LAL, destroy some amino acids and change the electrophoretic pattern. These findings were taken into consideration when planing the final process. Due to these potentially damaging changes, any extraction process should avoid the use of high pH and high temperatures until the protein changes are thoroughly understood.

#### 5.5 SELECTION AND TESTING OF THE TWO STAGE EXTRACTION PROCESS

A flow-chart of the final two stage process is shown in Fig. 3. The selection was based on the results obtained throughout this work. To test the proposed process, four batches were prepared according to the suggested flow-chart. The process produced three fractions: the liquid fraction (supernatant) resulting from centrifugation at the end of the first stage; liquid fraction (supernatant) resulting from the alkaline extraction of the solids portion, from the first step; and the solid residue after alkaline extraction (meal). Chemical composition of these fractions is shown in Table 16 and Table 17.

The results in Table 17 show that the first step extraction at the natural pH extracted about 52% of the total oil. Because the extraction took place at a high



Table 16 Yield and chemical composition\* of freeze-dried rapeseed fractions from the two stage extraction

Components	Units	Fractions			Rapeseed raw material
		First extraction	Second extraction	Solid residue	
		Supernatant	Supernatant		
Weight	gm	65.1	50.3	75.6	200
Oil	%	64.9	52.1	16.0	42.3
Protein (Nx6.25)	%	29.8	69.4	32.4	41.0
Crude fibre	%	1.1	7.9	22.1	13.5
Glucosinolates	mg/gm	3.4	0.7	0.0	2.9
Total ash	%	8.8	7.9	10.3	7.5
Lysinoalanine	ppm	0.0	0.0	0.0	0.0
Free Fatty acids	% oil	0.5	0.5	0.5	0.5

\*Average of at least two replicates. Except lines 1 and 2 all data were obtained for oil free samples.



Table 17 Percentage of extracted oil, protein, glucosinolates and fibre in fractions obtained during the two step extraction.

Component extracted	Fractions			
	First extraction		Second extraction	
	Supernatant	Supernatant	Solid residue	
	<div><div>&lt;</div><div>% of total</div><div>&gt;</div></div>			
Oil	52.5	32.5	15.0	
protein	15.5	37.9	46.6	
Glucosinolates	38.9	5.3	00.0	
Fibre	1.6	11.7	86.7	





temperature (80°C), most of the extracted oil was in the form of free oil, which makes it easy to separate the oil from the liquid fraction. The first step also removed 38.9% of the total glucosinolates which account for about 88% of the glucosinolates not destroyed as a result of the heat treatment. Nitrogen losses in the first extract were about 15.5% presumably mainly non-protein nitrogen.

The second extraction of the solids produced from the first extraction was conducted at pH 11 at 25°C for 30 minutes and resulted in further extraction of more than 32% of the total oil and more than 37% of the total nitrogen in the liquid fraction. Removal of the oil from the freeze-dried second liquid fraction produced protein concentrate with a protein content of more than 69%. The protein concentrate was of a light color as shown in the photo in Fig. 22. It contained no lysinoalanine, while the glucosinolates content was greatly reduced (Table 16). Further reduction could be anticipated if the extracted emulsion were broken and the precipitated protein separated from the supernatant containing the soluble glucosinolates. The solid residues contained about 22% crude fibre which constituted more than 86% of the total fibre in the original rapeseed sample. It also contained about 15% oil. This may be considered high when compared with current industrial meals obtained by prepress-solvent extraction process. However, this percentage can be reduced with the introduction of more suitable equipment. Neither





Fig. 22. Color of freeze-dried fractions produced from the two stage extraction.

- a. Liquid fraction from the first extraction
- b. Liquid fraction from the second extraction
- c. Solid fraction (meal)
- d. Commercial meal



glucosinolates nor lysinoalanine were found in the solid residue.

The above results confirm our earlier findings, especially regarding the oil yield in the first extraction and the quality of the produced protein.





## 6. SUMMARY AND CONCLUSION

The purpose of this work was to investigate an aqueous process for simultaneous recovery of oil and protein and to suggest a technique, producing three main products: good quality oil, good quality protein and meal.

The suggested technique consisted of two steps. In the first step we attempted to extract as much as possible of the oil and glucosinolates while maintaining the nitrogen losses in the aqueous extract at minimum. In the second step, the residues left from the first extraction are processed under alkaline conditions in order to extract as much as possible of both oil and protein in the liquid fraction.

Although the suggested two stage procedure is meant to be used as a complete operation, it could be feasible to use the first extraction step on its own right to replace the first part of the conventional pre-press solvent method which includes heating, crushing, cooking and pressing. For this reason the advantages and disadvantages of the two steps can be considered independently.

### 6.1 AQUEOUS EXTRACTION OF THE FULL-FAT RAPESEED

Some parameters affecting the aqueous extraction were investigated to obtain acceptable oil yield and percentage of free oil, and to limit nitrogen losses in the liquid fraction. Economical implications were taken into



consideration.

The selected extraction conditions are shown in Fig. 3. The conditions of the extraction were simplified to limit energy consumption and to minimize the extraction time. The procedure employed wet heat treatment for two minutes to deactivate the enzymes and insolubilize the proteins in the ground rapeseed. The extraction was conducted at the natural pH of the rapeseed, thus eliminating the need for pH adjustment and for the use of alkali or acid. Mixing was performed at 2000 rpm for 10 minutes. This is a very short time compared with any other process. The temperature of the extraction was quite high (80°C) in order to produce the highest percentage of free oil and to enhance the removal of glucosinolates. The advantages of this suggested first step extraction are:

- a. The method is in fact a simple washing of the oil from the ground and mixed seed. The equipment needed is simple and inexpensive.
- b. This simple washing extracts about 52% of the total oil. The produced oil is of good quality, is mostly in free state and does not need degumming. The oil yield can be further improved with optimization of the unit operations involved.
- c. Washing removes the majority of the glucosinolates which are not destroyed by the heat treatment. It might also remove some undesirable compounds which have adverse effects on animal health and limit the potential for obtaining food grade products. Most of the solids removed in the liquid





fraction were described by Rutkowski and Kozłowska (1979) as "antinutritional factors". These include some non-protein nitrogen compounds, oligosaccharides, pigments, phenols and some phytins. The oligosaccharides such as stachyose and raffinose are frequently claimed to have intestinal gas-forming properties (Theander and Aman, 1974). Pigments and phenols are linked to discoloration of rapeseed protein when extracted under alkaline conditions (Rutkowski and Kozłowska, 1979). Phytin also is known to be harmful (Jones, 1979., Ohlson and Anjou, 1979).

d. The treatment in boiling water for two minutes will destroy the antitryptic agents, which are highly sensitive to the effect of temperature (Rutkowski and Kozłowska, 1979).

The disadvantages of this treatment may include the losses of nitrogen and other solids in the aqueous fraction. However, earlier studies by Lo and Hill (1972) indicated that 47% of the nitrogen lost in the liquid fraction is in the form of non-protein nitrogen. According to Rutkowski and Kozłowska (1979), non-protein nitrogen is composed of peptides, free amino acids, the products of the incomplete synthesis or hydrolysis of protein, nucleic acids, glucosinolates, ammonia, nitrogen and other nitrogen-containing substances. The contents of peptides and free amino acids in non-protein nitrogen of rapeseed meal amounts to 7.2% and 13.3% respectively, or 2.4% of the meal weight (Rutkowski and Kozłowska, 1979). Out of this group of





compounds, only free amino acids have some value as food constituents. If desired, nitrogen losses in the liquid could be recovered using the industrial ultrafiltration technique (Manak *et al.*, 1980, Lawhon *et al.*, 1981), or reverse osmosis (Von Bockelmann *et al.*, 1974). Also the liquid fraction could be used as a medium for the growth of some microorganisms (Staron, 1970, 1974). Another possibility that could be investigated is the use of the liquid fraction for irrigation and as a fertilizer especially in the southern parts of Alberta. The loss of solids in the liquid fraction which resulted from the simple washing applied in this work is in line with the losses in other techniques using leaching or diffusion to remove glucosinolates from crushed or intact seeds. However, our technique takes much less time than the five hours needed for leaching (Anjou *et al.*, 1978) or 1 to 5 hour for diffusion (Kozłowska *et al.*, 1979). The latter techniques use a large volume of water when compared with our treatment.

The solid residue obtained after washing was found to contain between 36 to 40% moisture. The residue could possibly be dried and extracted by hexane to produce oil and good quality meal, or it can be used for the second, alkaline extraction step to produce oil, protein and meal.



## 6.2 THE SECOND EXTRACTION STEP: ALKALINE EXTRACTION.

The alkaline extraction step was intended to extract as much as possible of the remaining oil and protein from the solid fraction obtained by the first extraction.

The factors affecting the yield and the quality of the oil and the protein were investigated. The results showed that in order to maximize the protein solubility of heated rapeseed, extraction should take place at high pH and high temperature. However, the high pH and high temperature (particularly pH 11 and 70°C) were found to introduce undesirable changes, such as protein discoloration which increased with the severity of the treatment, oil hydrolysis, production of lysinoalanine and the destruction of some essential amino acids. No harmful effect on either oil or protein was found at low temperatures even at high pH. Therefore, alkaline extraction at pH 11 and 25°C for half an hour was selected. The latter treatment compromised the protein extractability, however, it produced a protein concentrate with good quality. This treatment left a portion of the protein in the meal which was considered desirable in order to maintain some quality of the meal at least for feeds.

Using the above treatment with the solid residue from the first extraction, we were able to extract additional 32.5% of the total oil and more than 37% of the total protein in the liquid fraction. Removal of oil from the dried liquid fraction by solvent produced protein





concentrate with a protein content of more than 69%. The protein concentrate was of a light color and contained no lysinoalanine. Glucosinolates content was 0.7 mg/gm., however, further reduction would be expected if the extracted emulsion was broken and the precipitated protein was separated from the supernatant containing the soluble glucosinolates. This also would produce protein concentrate with more protein and less soluble carbohydrates.

The solid residues of the second extraction (the meal) contained about 15.0% of the total oil and more than 46% of the total protein. The meal had a high content of fibre (22% on the fat-free basis) which might limit its use for swine and poultry because of the low metabolizable energy value (Clandinin *et al.* 1978). The relatively high oil losses in the meal should be reduced by improvements in the separation step. It is important to mention that crude oil produced by the prepress-solvent extraction process is washed by water to remove the gums. The gums together with some oil are usually added to the meal, thus effectively increasing the amount of oil lost in the meal to values which might be comparable with our results.

The meal was found to have no glucosinolates. The problem of breaking the emulsion resulting from the alkaline extraction to produce oil and protein was not investigated in this work, however, it should be technically feasible. Sugarman (1956) patented a method which involves the alkaline extraction of oilseeds to produce a liquid fraction





and a meal. The patent includes an emulsion breaking step to produce oil and protein.

The precipitation of the alkali-extracted protein was studied by many workers. In order to use the process in industry, countercurrent extraction should be applied along with precipitation at more than one step. El-Nockrashy *et al.* (1977) extracted as much as 94% of the total rapeseed nitrogen using a countercurrent procedure and two precipitation steps at pH 6 and 3.6. The same approach was followed by Yang *et al.* (1978). A counter-current procedure would make it possible to control and limit the amount of water used.

Because of the undesirable effect of phytic acid (Rutkowski and Kozłowska, 1979; Ohlson and Anjou, 1979; Jones, 1979), the precipitation should be accomplished in a multiple step process, which would include one precipitation step at acidic conditions where the phytates are at maximum solubility (Gillberg and Tornell, 1976). A rapeseed protein product with no or very little glucosinolates, phytates and other antinutrient factors would be indeed a valuable product with many possible uses competing with the currently used soybean.



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